

A Thesis Submitted for the Degree of PhD at the University of Warwick

Permanent WRAP URL:

<http://wrap.warwick.ac.uk/158972>

Copyright and reuse:

This thesis is made available online and is protected by original copyright.

Please scroll down to view the document itself.

Please refer to the repository record for this item for information to help you to cite it.

Our policy information is available from the repository home page.

For more information, please contact the WRAP Team at: wrap@warwick.ac.uk

Consequences, Challenges and Future Solutions in Combat Lower Limb Trauma

by

Philippa Mary Bennett

A thesis submitted in partial fulfilment of the requirements for the
degree of
Doctor of Philosophy in Medicine

University of Warwick Department of Medicine
January 2020

Contents

	Page
Contents	1
List of figures	5
List of tables	7
Dedication	9
Acknowledgements	11
Declaration	13
Summary	15
List of abbreviations	17
Section 1 Introduction and rationale	19
1.0 Introduction	21
1.1 Rationale	21
References	23
Section 2 Relationship between works	25
2.0 Introduction	27
2.1 Military trauma care	27
2.1.1 <i>Deployed medical care</i>	27
2.1.2 <i>Trauma registries</i>	28
2.1.3 <i>Epidemiology of combat injuries</i>	29
2.1.4 <i>Follow-up of military casualties</i>	29
2.2 Methodology	30
2.2.1 <i>Ethical approval</i>	30
2.2.2 <i>New injury severity score (NISS)</i>	31
2.2.3 <i>Outcomes</i>	31
2.3 Clinical studies	34
2.3.1 <i>Unilateral lower limb amputation</i>	34
2.3.2 <i>Open femur fractures</i>	35
2.3.3 <i>Open tibia fractures</i>	38
2.3.4 <i>Hindfoot fractures: identification of cohort & early outcomes</i>	40
2.3.5 <i>Hindfoot fractures – establishing functional outcomes</i>	42
2.4 Systematic review of preclinical therapies to prevent or treat fracture non-union	45
2.5 Summary	51
References	53
Section 3 Contribution to original knowledge	57
3.0 Introduction	59
3.1 Clinical studies	59
3.1.1 <i>Unilateral lower limb amputation</i>	59
3.1.2 <i>Open femur fractures</i>	60
3.1.3 <i>Open tibia fractures</i>	61
3.1.4 <i>Hindfoot fractures</i>	61
3.2 Systematic review of preclinical therapies to prevent or treat fracture non-union	62
References	64

Section 4 Methodological critique and reflection	67
4.0 Introduction	69
4.1 Research training	69
4.2 Designing research	70
4.3 Methodological weaknesses	71
4.3.1 Registries	71
4.3.2 The use of controls	71
4.3.3 Follow-up	72
4.3.4 Outcomes	72
4.4 Generalisability	73
4.5 Future research	74
References	76
Confirmation of contribution letters	79
Author's bibliography	93
Appendices	99
1. Paper one: unilateral lower limb amputation	101
2. Paper two: open femur fractures	107
3. Paper three: open tibia fractures	113
4. Paper four: hindfoot fractures – identification of cohort & early outcomes	119
5. Paper five: hindfoot fractures – establishing functional outcomes	127
6. Paper six: systematic review of preclinical therapies to prevent or treat fracture non-union	135

List of Figures

Figure	Legend	Page
2.1	Schematic of deployed medical care for UK armed forces including clinical timelines	28
2.2	Plot showing the mean SF-36 PCS by level of amputation	35
2.3	Bar graph showing bone loss and outcome	37
2.4	Clinical image showing significant soft tissue and periosteal stripping following open tibia fracture sustained following IED explosion	38
2.5	Radiograph showing significant soft tissue and periosteal stripping following open tibia fracture sustained following IED explosion	38
2.6	Graph showing the variation in AAOS F&A score depending on presence of key variables on initial injury	44
2.7	Graph showing the correlation between AAOS F&A score and SF-12 score by Spearman's correlation coefficient test	44
2.8	Graph showing the comparison between SF-12 PCS outcome scores for patients with reconstructed hindfoot injuries, and those requiring amputation following attempted reconstruction	44
2.9	Graph showing the variation in SF-12 PCS scores for those with reconstructed hindfoot injuries depending on presence of key variables in initial injury, compared with those patients requiring delayed amputation following attempted reconstruction	44
2.10	Schematic comparing rates of fracture non-union in military casualties to those seen in civilian series	46
2.11	Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRSIMA) flow-diagram for exclusion of papers included in systematic review	50

List of Tables

Table	Legend	Page
2.1	Search terms for clinical studies	30
2.2	Registration numbers for clinical studies	31
2.3	Summary of clinical papers	33
2.4	Grading system for bone loss	37
2.5	Indications for amputation at 18 months	41
2.6	Indications for amputation	43
2.7	Fracture burden by individual	46
2.8	Summary of study inclusion and exclusion criteria	49

JPB

For being the start, and now being everything

My parents

No daughter could have asked for more

Acknowledgements

Surg Cdr JG Penn-Barwell RN who conceived these studies, and whose constant guidance and advice saw them through to their conclusions.

Surg Capt R Rickard RN for navigating open tibial fractures with us, and for his expert plastic and reconstructive surgical advice.

Surg Capt M Midwinter CBE RN (Rtd) for his support and guidance during the earliest stages of this work.

Members of the Severe Lower Extremity Combat Trauma (SeLECT) study group: **Gp Capt Ian Sargeant OBE, Prof Sir Keith Porter KBE, Gp Capt Jon Kendrew, Lt Col Tom Rowlands, Lt Col Al Mountain and Dr Deb Mortiboy** who collectively provided the clinical care for these patients, and whose views and experience in the management of combat injury formed the basis of these studies.

Dr Danai Bem and Janine Dretzke of the Institute of Applied Health Research, University of Birmingham for providing the expertise that ensured the systematic review was conducted correctly and concluded.

Dr David Ellard and Dr Peter Wall for their supervision of this thesis, and guiding me through this process.

Declaration

All six of the studies which form the basis of this submission have been published in peer reviewed journals. While these studies were the result of collaborative research I can confirm that this thesis is my own work. Confirmation of my individual contribution to each study can be found within the signed letters from my co-authors contained within this submission.

I can confirm that no part of this thesis has been submitted for a degree at any other University.

Summary

The British Military was engaged in over a decade of conflict during the wars of Iraq and Afghanistan. The enemy's use of Improvised Explosive Devices (IEDs) saw traumatic lower limb amputation representing one end of the spectrum of injury severity, with multiple fractures of the femur, tibia and hindfoot also seen.

The surgical approach to both traumatically amputated and severely injured limbs evolved over the course of the conflicts. Amputations were debrided within the zone of injury in an attempt to preserve limb length, an approach which required multiple surgical episodes. Patients whose fractures were deemed suitable for attempts at limb reconstruction underwent multiple orthopaedic and reconstructive procedures with the assumption that they would have superior functional outcomes than if they underwent amputation.

These changing surgical strategies and a realisation of the uncertainty of the optimum approach presented a "*could* versus *should*" paradigm of limb reconstruction. Just because it was thought a limb *could* be reconstructed, *should* it be? What would give patients the optimal functional outcome? Limb reconstruction, or amputation?

To answer this question my research characterised the patterns of lower limb injuries seen following combat, presented functional outcomes, identified causes for poor outcomes and systematically investigated potential solutions. This research challenges the accepted definition of "success" following severe hindfoot trauma, and establishes the specific injury patterns that are associated with a worse functional outcome than those patients undergoing unilateral lower limb amputation.

My analysis of lower limb combat injuries revealed that rates of fracture non-union in military patients are higher than those seen following similar civilian injuries. Having published the very first systematic review into preclinical therapies for fracture non-union, the research highlights the alarmingly poor state of research, and calls for international collaboration and standards to more rapidly identify therapies that may successfully treat this devastating condition.

List of Abbreviations

Abbreviation	
AAOS F&A	American Academy of Orthopaedic Surgeons Foot and Ankle questionnaire
AIS	Abbreviated Injury Scale
AO	Arbeitsgemeinschaft für Osteosynthesefragen
BAPRAS	British Association of Plastic, Reconstructive and Aesthetic Surgeons
BATLS	Battlefield Advanced Trauma Life Support
BOA	British Orthopaedic Association
DMS	Defence Medical Services
IED	Improvised Explosive Device
IQR	Interquartile range
ISS	Injury Severity Score
JTTR	Joint Theatre Trauma Registry
KD	Knee disarticulation
MCS	Mental component score
NATO	North Atlantic Treaty Organisation
NISS	New Injury Severity Score
NJR	National Joint Registry
OI	Osseointegration
OR	Odds ratio
OTA	Orthopaedic Trauma Association
PCS	Physical component score
PICO	Population, intervention, comparator, outcome
PRISMA	Preferred reporting items for systematic review and meta-analysis
PROM	Patient Reported Outcome Measures
RCDM	Royal Centre for Defence Medicine
RCT	Randomised controlled trial
SF-12	Short Format-12 questionnaire
SF-36	Short Format-36 questionnaire
TFA	Transfemoral amputation
TNC	Trauma Nurse Coordinator
TTA	Transtibial amputation
UK	United Kingdom
UKDS	United Kingdom Defence Statistics

SECTION 1

INTRODUCTION AND RATIONALE

1.0 Introduction

From the invasion of Iraq in 2003 to the cessation of hostilities in Afghanistan in 2014 the United Kingdom (UK) military was involved in over a decade of high-intensity combat operations. During this time 2,792 British personnel were injured or killed during service, of which 608 (22% of all casualties) were fatalities¹.

The duration of operations and the enemies' use of Improvised Explosive Devices (IEDs) led to advancements in the design of body armour and the development of new resuscitation techniques. As such, it was speculated that casualties were surviving who previously would have succumbed to their injuries, with the corollary that surgeons encountered injuries of a severity not previously seen².

As surgeons developed new techniques to manage these severe injuries, a requirement to prove the assumed superiority of their approach became apparent. In the series of papers submitted for this PhD I describe work that aimed to characterise the patterns of lower limb injuries seen following combat trauma, examine the surgical management of these injuries, measure early and medium-term functional outcomes, identify causes for poor outcomes and systematically investigate potential solutions.

1.1 Rationale

Amputation has historically been the mainstay of treatment for open fractures of the lower limb³. Along with very limited orthopaedic and plastic reconstructive knowledge, military surgeons recognised that the risk of death from infection mandated amputation, "when part of a Limb is carried away, or the Bones so shattered"^{4,5}. As anaesthesia was developed and the understanding of orthopaedic and plastic reconstructive surgery improved the option to reconstruct and 'salvage' limbs became increasingly viable.

As limb reconstruction became an alternative to amputation, its success was simply measured in the avoidance of amputation. However, the appropriate definition of successful limb reconstruction is considerably more nuanced. From the patient's perspective they require a functional, pain-free limb, which surgically translates into united fractures without infection and with healed, closed wounds that are sensate and comfortable. Adjacent joints should be mobile and pain free.

As British surgeons were seeing large numbers of casualties from Iraq and Afghanistan with severe lower limb trauma it was clear that modern surgical practices ensured limb reconstruction was an option in injuries that previously would have resulted in amputation. However, such decisions commit patients to multiple surgeries and prolonged rehabilitation programmes with no guarantee of "success". Although it was presumed that patients "did better" with limb reconstruction rather than amputation there was no evidence to support this assumption.

The requirement to provide the data on which to base surgical decision-making forms the basis of the works submitted for this PhD. The only way to determine whether those undergoing limb reconstruction "did better" than those with an amputation was to identify each cohort, then establish the clinical outcomes of each group. Only by evaluating surgical practice and identifying complications can surgeons be sure they are providing "the best" care for their patients.

In the next chapter I will provide an overview of each of the studies submitted as part of this PhD, demonstrating how they are inter-related and how they each contributed further information to the limb reconstruction versus amputation debate. In the following chapter I will emphasise the original knowledge they have contributed to the field of orthopaedic trauma surgery. The final chapter provides a critique of the methodologies used in each of my studies, with reflection on what I have learnt while doing this research and how I would conduct further research in the future.

References

1. Penn-Barwell JG, Roberts SA, Midwinter MJ, Bishop JR. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003–2012. *Journal of Trauma and Acute Care Surgery* 2015; **78**(5): 1014-20.
2. Kelly JF, Ritenour AE, McLaughlin DF, et al. Injury Severity and Causes of Death From Operation Iraqi Freedom and Operation Enduring Freedom: 2003–2004 Versus 2006. *Journal of Trauma and Acute Care Surgery* 2008; **64**(2): S21-S7.
3. Kirkup JR. A history of limb amputation: Springer Science & Business Media; 2007.
4. Moyle J. Chirurgus Marinus; Or, The Sea-chirurgion. London: Tracey; 1693.
5. Shuster S. Painless amputation: history of a discovery that wasn't made. *Bmj* 2009; **339**: b5202.

SECTION 2

RELATIONSHIP BETWEEN WORKS

2.0 Introduction

The aim of this body of work was to establish the outcomes of patients with severe lower extremity trauma sustained in combat. I also aimed to try to determine which treatment strategy - amputation or limb reconstruction – offered the best outcome for patients following severe lower extremity combat trauma.

By dividing lower limb injuries into three main anatomical regions (femur, tibia, hindfoot) the clinical outcomes for each can be considered and compared to those established in patients with lower limb amputation.

2.1 Military Trauma Care

2.1.1 *Deployed medical care*

The delivery of medical care from the point of wounding on the battlefield to the patient's discharge from rehabilitation services involves multiple healthcare professionals delivering care at numerous stages and in numerous locations. During combat operations in Iraq and Afghanistan medical care was delivered in line with Clinical Guidelines for Operations and North Atlantic Treaty Organisation (NATO) doctrine^{1,2}.

Current Defence Medical Services (DMS) practice is that immediate care on the battlefield is delivered by combat medical technicians using the principles of Battlefield Advanced Trauma Life Support³ (BATLS). Casualties are then evacuated to more advanced treatment facilities where care is delivered by doctors, prior to onward repatriation to a single facility at the Royal Centre for Defence Medicine (RCDM) in Birmingham, UK. Rehabilitation services are provided at the Defence Medical Rehabilitation Centre Headley Court near Epsom in Surrey, UK (**Figure 2.1**).

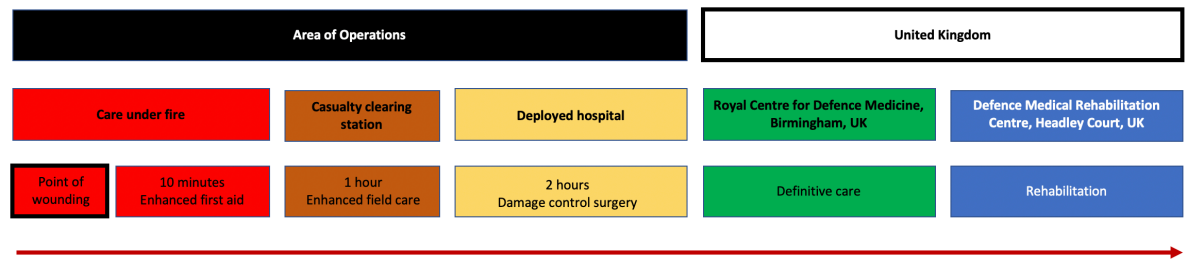


Figure 2.1: schematic of deployed medical care for UK armed forces including clinical timelines

2.1.2 Trauma registries

The first modern military registry of trauma care was established by Brigadier Tim Hodgetts during peace-keeping operations in Kosovo in 1999⁴. This was the basis of the Joint Theatre Trauma Registry (JTTR), designed specifically to measure the care given to combat casualties by including statistical tools to measure the standard of care provided⁵. The JTTR is administered by the UK Defence Statistics (UKDS) agency and was in place on the invasion of Iraq in March 2003⁶.

Data entry into JTTR is done by specially trained research nurses known as Trauma Nurse Coordinators (TNCs). TNCs prospectively gather data on; 1) all those killed immediately; 2) those whose injuries trigger a trauma alert on arrival at a deployed hospital facility; 3) those whose injuries subsequently require repatriation to RCDM. A trauma alert is a systemised notification method of ensuring essential personnel and resources are rapidly available to resuscitate, diagnose and treat severely injured casualties⁷. One team of TNCs is based in the deployed hospital with another team in RCDM.

In addition to demographic data and details on the mechanism of injury, the JTTR codifies injuries using the Abbreviated Injury Scale (AIS) and also includes physiological data such as vital signs and conscious level. The AIS is an internationally used system of defining injury severity throughout the body based on anatomical regions^{8,9}. Although some details on injury treatment are captured by JTTR this predominantly focuses on initial management in the Emergency

Department such as the administration of blood products. The only outcome recorded is mortality, with no detail on surgery or rehabilitation.

2.1.3 Epidemiology of combat injuries

Injuries sustained on the battlefield differ from those seen in civilian practice. They are typically the result of an intent to wound or kill through the use of explosives and high-energy gunshot wounds. As service personnel are normally wearing body armour and helmets when engaged in combat, the relative distribution of injuries sustained also differs from that seen in civilian casualties.

Injuries of the lower limbs were the predominant injury pattern seen throughout the conflicts in Iraq and Afghanistan¹⁰. The use of IEDs by insurgents characterised these conflicts¹¹, and were responsible for over 45% of casualties¹². Although bilateral lower limb amputation as a result of IEDs was often seen as the “signature injury” of these conflicts, this was at one end of a spectrum of injuries, which included fractures of a range of severity of all bones of the lower limb.

2.1.4 Follow-up of military casualties

Regardless of where in the world military personnel are injured they will be repatriated to RCDM, Birmingham, UK. Although acute injuries are managed here it is not uncommon for patients to request that routine follow-up is transferred to hospitals nearer their units to facilitate further appointments. The hospital records from RCDM are therefore accurate in providing information on individuals’ acute care but cannot be relied upon for prolonged follow-up beyond 18 months. Similarly, although patients who remain in the military will access Defence Primary Healthcare, those discharged from the Armed Forces will have their primary care transferred to a civilian GP, and oversight of follow-up is subsequently lost.

2.2 Methodology

Six papers (**Appendix 1 – 6**) constitute this PhD. The first five of these are clinical papers which share similar methodologies, an overview of which will be provided here. The aim of the clinical papers was:

1. To identify British military personnel sustaining specific lower limb injury patterns during combat Operations in Iraq and Afghanistan;
2. Measure and draw inferences on pain, function and quality of life following lower limb injury using Patient Reported Outcome Measures (PROMs).

I identified patients through coding searches of JTTR, as outlined in **Table 2.1**. I used patient hospital numbers to search the clinical and radiographical records of individuals at RCDM as per each study's inclusion and exclusion criteria.

Table 2.1 Search terms for clinical studies

Study	JTTR ^a search codes
Unilateral lower limb amputation	Unilateral lower limb amputation
Open femurs	Bony injuries of hip, femur or knee
Open tibias	Bony injuries of knee, tibia or ankle
Hindfoot fractures*	Bony injuries of foot, ankle, fibula, tibia, talus, calcaneus or other tarsal bones

^a Joint Theatre Trauma Registry

*Both hindfoot fracture studies used the same cohort of patients, identified through the same JTTR search

2.2.1 Ethical approval

I registered all studies with Joint Medical Command and Royal Centre for Defence Medicine (RCDM) as per **Table 2.2**.

Table 2.2 Registration numbers for clinical studies

Study	Registration number
Unilateral lower limb amputation	RCDM/Res/Audit/1036/12/0385
Open femurs and open tibias	RCDM/Res/Audit/1036/12/0137
Hindfoot injuries	RCDM/Res/Audit/1036/15/0439

2.2.2 New injury severity score (NISS)

This is used throughout JTTR as a measure of injury severity and was collected alongside demographic data of those injured. NISS is calculated from the 2005 military edition of the Abbreviated Injury Scale (AIS) coding manual¹³ described in section 2.1.2 above, and is used in preference to the Injury Severity Score (ISS)¹⁴. Where ISS only measures the most severe injuries from separate body regions, NISS measures the most severe injuries even if they occur in the same region and has been found to more accurately quantify combat trauma^{15,16}. A NISS of more than 15 is the commonly used definition of major trauma; the maximum is 75, which is deemed as not survivable.

2.2.3 Outcomes

The following outcomes were captured as part of this research:

1. Revision surgery

This was captured from patient notes and review of radiographs. Patients were also asked about unplanned revision surgery when they were phoned for follow-up.

2. Conversion to amputation

This was captured from patient notes and review of radiographs. Patients were also asked about delayed amputation when they were phoned for follow-up.

3. Deep infection

This was defined as infection requiring surgical treatment and was captured by a review of microbiology results correlated with surgical episodes.

4. Fracture union

This was assessed by review of clinical letters and radiographs. Bony union was defined as cortical contact of three or more of four cortices on two tangential radiographs.

5. Quality of life

I used three separate follow-up questionnaires in the clinical studies to establish PROMs; 1) Short-format 36 (SF-36); 2) Short-format 12 (SF-12); 3) the American Academy of Orthopaedic Surgeons Foot & Ankle (AAOS F&A) outcome questionnaire.

The United Kingdom-adapted second version of the SF-36 tool was developed as a non-specific measure of the impact of illness or disability on quality of life¹⁷. Although it is not specific for amputees it has the ability to differentiate between the physical and mental components of injury recovery and has been used in outcome studies looking at both amputation and trauma^{18,19}. Scores range from 0 (lowest level of health) to 100 (highest level of health).

The United Kingdom-adapted second version of the SF-12 questionnaire similarly ranges from 0-100²⁰. In an attempt to reduce responder burden it asks 12 questions rather than 36 and has been shown to correlate closely with SF-36²¹.

6. Joint specific quality of life

I chose the AAOS F&A questionnaire for follow-up of patients with hindfoot injuries as it does not require clinical assessment and can be administered over the telephone. It has been shown to correlate well with other quality of life outcome measures^{22,23}.

Table 2.3 Summary of clinical papers

	Appendix	Study period	Number of patients	Age (median (IQR))	NISS ^a (median (IQR))	Follow-up		
						Method	Time	Numbers (%)
Unilateral lower limb amputation	1	March 2004 – March 2010	48	23 (21-26)	24 (17-34)	SF-36	Mean 40 months (25-75, SD 16)	39/48 (81)
Open femur fractures	2	March 2003 – December 2012	48	23 (20-29)	22 (9-34)	Clinical / radiographic	Median 37 (IQR 19-53)	47/48 (98)
Open tibia fractures	3	April 2006 – September 2010	49 patients with 57 fractures (8 bilateral)	26 (19-43)	22 (4-45)	Clinical / radiographic	12 months	44 patients, 52 tibiae (52/57, 91)
Hindfoot fractures	4	January 2003 – December 2014	114 patients with 134 fractured hindfeet (20 bilateral)	26 (21-28)	12 (8-17)	Clinical / radiographic	18 months	92 patients (81), 114 hindfeet (85)
	5	January 2003 – December 2014	114 patients with 134 fractured hindfeet (20 bilateral)	26 (21-28)	12 (8-17)	SF-12 AAOS F&A ^b	60 months (IQR 52-80)	90 fractures (90/134, 67)

^a New Injury Severity Score (NISS)^b American Academy Orthopaedic Surgeons Foot & Ankle (AAOS F&A) outcomes questionnaire

2.3 Clinical Studies

I identified four separate injury cohorts; 1) unilateral lower limb amputees; 2) open femur fractures; 3) open tibia fractures; 4) hindfoot fractures. I established specifics of the injuries and obtained follow-up as detailed in **Table 2.3** and the sections that follow.

2.3.1 Unilateral lower limb amputation

Appendix 1

Bennett, P. M., et al. (2013). "Unilateral lower limb loss following combat injury: medium-term outcomes in British military amputees." *Bone Joint J* 95-B(2): 224-229.

Aims

In this paper I aimed to identify all patients sustaining a traumatic amputation of the lower limb and determine their medium-term outcomes by level of amputation. The outcomes I obtained from follow-up of these patients would provide the benchmark against which other injuries could be compared: this would allow me to determine the relative functional outcomes of those undergoing limb reconstruction versus amputation.

Methods

Patients were included if:

1. They underwent an amputation at the ankle or more proximally;
2. Their amputation occurred within the first seven days of injury.

I divided patients into three cohorts depending on the level of amputation; 1) trans-tibial amputation (TTA, "below-knee"); 2) knee disarticulation (KD, "through knee"); 3) trans-femoral amputation (TFA, "above knee").

The threshold for statistical significance was set at $p < 0.05$.

Findings

Forty-eight patients met the inclusion criteria. Minimum two-year SF-36 scores were completed by 39 patients (81%) at a median of 40 months (25-75).

I found that the physical component score (PCS) of the SF-36 declined significantly with more proximal amputation levels ($p=0.01$, Kruskal-Wallis), as shown in **Figure 2.2**. However, I did not establish a significant difference between the trans-femoral and knee disarticulation cohorts when compared directly ($p=0.178$, Mann-Whitney).

I found that mental component scores (MCS) did not vary across groups ($p=0.014$, Kruskal Wallis).

2.3.2 Open femur fractures

Appendix 2

Bennett PM, et al. (2015). "The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period." *Bone & Joint Journal* **2015**(97-B): 842-846.

The rates of deep infection following open femur fracture has varied from 27% in the Second World War²⁴, to 12% during the Balkan conflict of the 1990s²⁵, to 31% in a contemporary series from the United States²⁶. If one of the indicators of successful surgical treatment as outlined in section 1.1 is united fractures without infection then surgeons have a responsibility to ensure that they are achieving this through their management techniques.

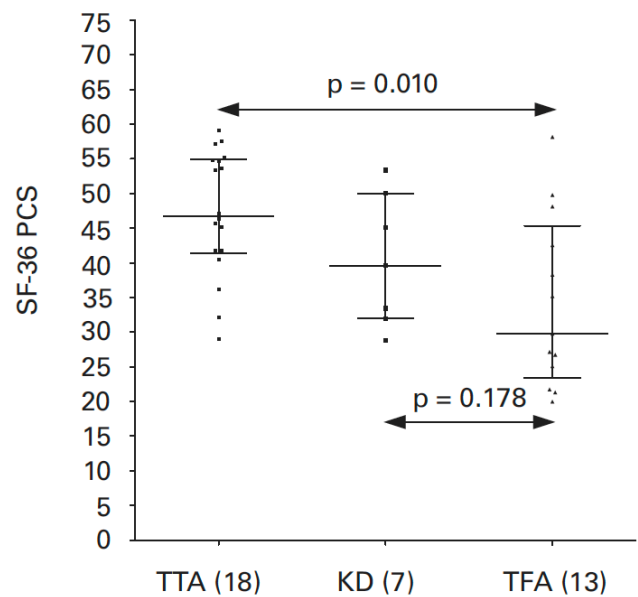


Figure 2.2. Plot showing the mean SF-36 PCS by level of amputation: TTA trans-tibial amputation, KD knee disarticulation, TFA trans-femoral amputation. The transverse lines represent the mean values and the error bars represent the standard deviation.

Aims

I aimed to characterise open femur fractures in survivors of the conflicts of Iraq and Afghanistan, establish rates of fracture union and identify factors associated with delayed fracture union.

Methods

The following patients were included:

1. British military survivors with AO type 32 open fracture of the femur²⁷;
2. Those with an ipsilateral lower limb amputation through or below the knee in the presence of a qualifying femur fracture;
3. Patients with more than 12 months' follow-up available.

Statistical significance was set at $p < 0.01$.

Findings

I identified forty-eight patients with 48 open fractures of the femur during the period of this study. No patient died following an isolated open femur fracture, a crude indicator of the improvements in care since the First World War.

It was possible for me to obtain complete follow-up with the use of clinical and radiographic records in 47 patients (98%) at a median of 37 months (IQR 19-53). Union of the fracture was achieved in 31 of these 47 patients (66%) without further surgical intervention.

Deep infection requiring surgical treatment occurred in four fractures (8%): all cases required further surgery before achieving union. Although meaningful statistical analysis of such small numbers is limited, I found no association between the presence of deep infection and the rate of union at 12 months ($p = 0.0102$, Fisher's exact test).

The degree of bone loss associated with the femoral fracture was also investigated in this study, and graded according to **Table 2.4**. I was able to measure bone loss radiographically in 44 patients, of which 27 (61%) had associated bone loss. I found that the degree of femoral bone loss was significantly associated with the requirement for further surgery ($p=0.00204$, Fisher's exact test), as shown in **Figure 2.3**. Two fractures failed to unite within the study period: both patients underwent trans-femoral amputation.

Table 2.4. Grading system for bone loss

Grade	Definition
0	None
1	Minimal , some bone loss but <1cm longitudinally around >50% of the circumference of the shaft and with some cortical contact
2	Moderate , bone loss between 1 and 2cm around >50% of the circumference of the shaft but with some cortical contact
3	Severe , bone loss >2cm around >50% of the circumference of the shaft but with some cortical contact
4	Segmental bone loss

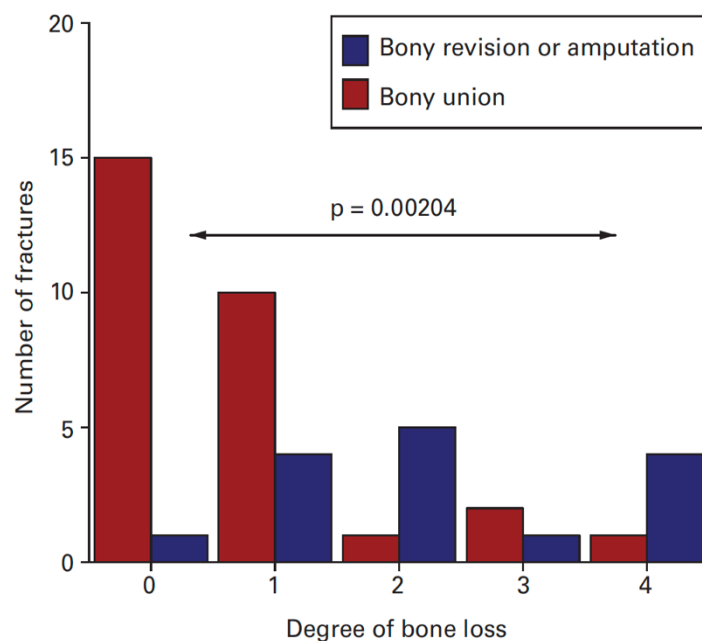


Figure 2.3. Bar graph showing bone loss and outcome. P-value according to Fisher's exact test across all groups is shown.

2.3.3 Open tibia fractures

Appendix 3

Penn-Barwell, J. G., et al. (2013). "Severe open tibial fractures in combat trauma: management and preliminary outcomes." *Bone Joint J* **95-B**(1): 101-105.

The superficial nature of the tibia and its poor blood supply make the management of open tibial fractures challenging, requiring specialist orthopaedic and plastic surgical care^{28,29}. When sustained in combat these injuries are usually the result of higher energy mechanisms such as military munitions and IEDs than those seen in civilian practice from road traffic collisions or falls. This difference manifests in the degree of tissue loss and periosteal stripping commonly seen in combat injuries, along with severe contamination requiring extensive debridement to prevent infection, as shown in **Figures 2.4 and 2.5**.



Figure 2.4 and 2.5. Clinical image and radiograph showing significant soft tissue and periosteal stripping following open tibia fracture sustained following IED explosion.

Aims

I aimed to characterize the pattern of severe open tibial fractures from the conflicts in Iraq and Afghanistan, and to identify factors that might be associated with poor outcome.

Methods

The following patients were included:

1. British military survivors with Gustilo-Anderson Grade III³⁰ AO type 42 open fracture of the tibia;
2. Those whose injuries did not require primary amputation of the limb within the first three surgical episodes.

I determined outcomes for this study at 12 months depending on whether the fracture had united at this point. I deemed that lack of union or unplanned revision of the fracture fixation was a marker of poor outcome, as was amputation after the third surgical episode.

Statistical significance was set at $p < 0.01$.

Findings

I identified 49 patients with 57 severe open tibial fractures. I obtained complete follow-up data for 44 patients and 52 tibiae at 12 months (44/49, 90%, 52/57, 91%). At this point, 26 of 52 tibiae (50%) had achieved union. Revision surgery had been required for 19 tibiae (19/52, 36%): seven (7/52, 13%) required amputation as it was felt that further attempts at salvage would be inappropriate.

I found that deep infection requiring surgical debridement occurred in the management of 12 of 52 fractures (23%), 11 of which occurred in fractures which failed to unite at 12 months. There was a statistically significant association between deep infection requiring surgical treatment and poor outcome ($p = 0.008$,

Fisher's exact test). Conversely to the findings of the open femur fracture study, I found that the degree of tibial bone loss was not associated with failure of fracture union at 12 months ($p=0.046$).

2.3.4 Hindfoot fractures – identification of cohort and early outcomes

Appendix 4

Bennett, P., et al. (2017). "Salvage of Combat Hindfoot Fractures in 2003-2014 UK Military." Foot & Ankle International **38**(7): 745-751.

Fractures of the hindfoot emerged from the conflicts of Iraq and Afghanistan as the greatest challenge to orthopaedic surgeons when considering if they could be salvaged³¹. In the civilian setting, the optimal management of intra-articular fractures of the calcaneum remains controversial^{32,33}; those sustained in the military are highly complex due to the blast wave from IEDs³⁴. Surgery aims to provide patients with a painless, plantigrade, functional foot, with united fractures and healed wounds.

The controversial management of these injuries can be distilled into two questions: *could* the injured limb be salvaged? And *should* it be?

Aims

I aimed to address the first of these questions, the “could” of hindfoot fractures. This question considers whether it is technically possible to reconstruct the injured hindfoot and obtain an outcome where the limb is free from infection and with all fractures united and wounds healed. For the study period in question the assumption of the treating surgical team was that hindfoot injuries should undergo limb reconstruction rather than amputation whenever feasible. Therefore, in this study I aimed to characterize the pattern of hindfoot injuries, define the early amputation and infection rate, and identify factors associated with poor early outcome.

Methods

The following patients were included:

1. British military survivors sustaining any fracture of the calcaneus or talus;
2. Those whose injuries did not require primary amputation of the limb within the first three surgical episodes.

Findings

I identified 114 patients with 134 hindfoot fractures: 20 patients sustained bilateral fractures. I was able to obtain 18-month follow-up for 92 patients (92/114, 81%) and 114 hindfeet (114/134, 85%). Statistical significance was set at $p < 0.05$.

Fifty cases of those I identified were open fractures (50/134, 37%). Thirty-six (36/114, 31%) fractures required unplanned revision surgery within the first 18-months following injury. I found that 19 limbs (19/114, 17%) required transtibial amputation when attempts at

Table 2.5. Indications for amputation at 18 months (n=19)

Indication	n
Aseptic nonunion / avascular necrosis	6
Deep infection	6
Pain despite radiographic union	5
Nonviable soft tissues	2

ongoing limb salvage and reconstruction were no longer deemed to be appropriate, the indications for which I have shown in **Table 2.5**.

Five patients whose calcaneal fractures were initially managed non-operatively underwent amputation within this 18-month period. On review of the notes I found two of these were for deep infection in open fractures, two for fracture non-union and one was required for chronic pain despite evidence of fracture union on radiographs.

I developed a binomial logistic regression model to analyse the requirement for amputation at 18 months while controlling for multiple variables. Using this model I showed that deep infection alone was associated with a significant requirement for amputation at 18 months ($p=0.23$).

2.3.5 Hindfoot fractures – establishing functional outcomes

Appendix 5

Bennett, P., et al. (2018). "Outcomes following limb salvage after combat hindfoot injury are inferior to delayed amputation at five years." *Bone and Joint Research* 7(2): 131-138.

Aims

I aimed to establish functional outcomes of patients with hindfoot fractures. I also aimed to identify injury features which, although limbs were “successfully” salvaged, led to a poorer recovery than individuals with a lower limb amputation.

Methods

For this study the cohort of patients with hindfoot fractures I identified in section 2.3.4 above were contacted by telephone. Those who consented to participate were assessed with the SF12 questionnaire; in addition, those who had retained their limb (i.e. had not subsequently required amputation) were also asked the AAOS F&A outcomes questionnaire.

Statistical analysis

I considered injury features identifiable at the time of wounding in a multivariate regression model. This allowed me to examine whether the variables were associated with a lower AAOS F&A score in those who retained their limbs. I used the Spearman’s correlation coefficient test to establish the relationship between the AAOS F&A score and SF-12 score. The threshold for significance was set at $p < 0.05$.

Findings

Of the 114 patients with 134 injured hindfeet I identified from the conflicts in Iraq and Afghanistan a total of 77 patients (77/114, 68%) were successfully contacted and consented to participate in the study. As 13 patients had bilateral injuries this provided me follow-up data for 90 injured limbs (90/134, 67%) with a median follow-up of 64 months (IQR 52-80).

Following attempts at hindfoot reconstruction, I found 28 limbs (28/90, 31%) subsequently underwent amputation at a median of 14 months (IQR 11-21) from time of injury. In 19 of these (19/28, 67%) pain was cited as the predominant reason to elect for amputation, with further detail provided in **Table 2.6**.

Table 2.6. Indications for amputation (n=28)

Indication	N (%)
Pain	19 (67)
Quality of life	4 (14)
Nonunion	2 (7)
Infection	2 (7)
Soft tissue breakdown	1 (3.5)

Negative Bohler's angle on initial radiograph

Coexisting fractures of talus and calcaneum

Fracture of tibial plafond in addition to hindfoot fracture

Box 2.1. Key injury variables associated with poor AAOS F&A score.

In the 62 cases where the limb was retained the median AAOS F&A score was 74 (IQR 61-88). Using the multivariable regression analysis I identified three key injury variables associated with a significantly poorer score, as shown in **Figure 2.6**. Of these patients the AAOS F&A score was significantly associated with the number of variables present ($p=0.0021$, Kruskal-Wallis) as shown in **Box 2.1**. Despite the reduction in AAOS F&A score I found injuries characterized by one or more of these key variables were not associated with an increased risk of amputation ($p=0.1717$, Fisher's exact test).

Of the 90 limbs followed-up, only one possessed all three of these key injury variables: this limb was amputated 20 months after injury due to the development of osteomyelitis.

I confirmed the positive correlation between AAOS F&A score and SF-12 (**Figure 2.7**).

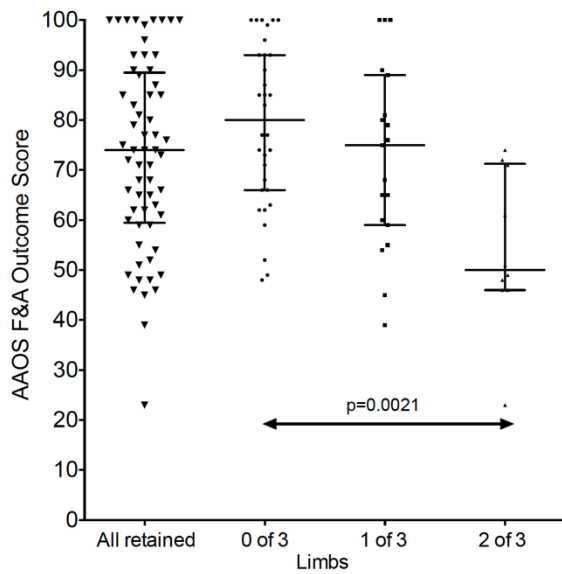


Figure 2.6. Graph showing the variation in AAOS F&A score depending on presence of key variables on initial injury.

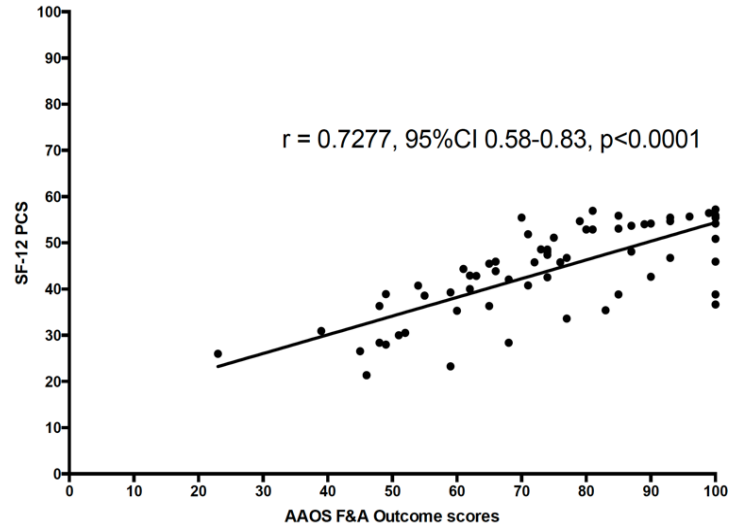


Figure 2.7. Graph showing the correlation between AAOS F&A score and SF-12 score by Spearman's correlation coefficient test.

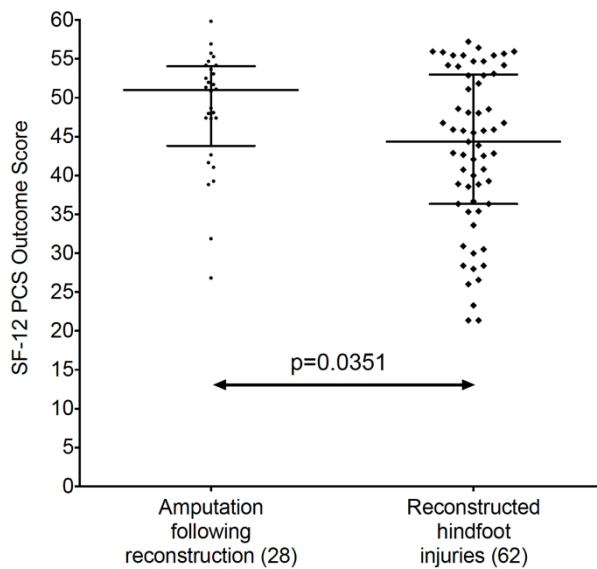


Figure 2.8. Graph showing the comparison between SF-12 PCS outcome scores for patients with reconstructed hindfoot injuries, and those requiring amputation following attempted reconstruction

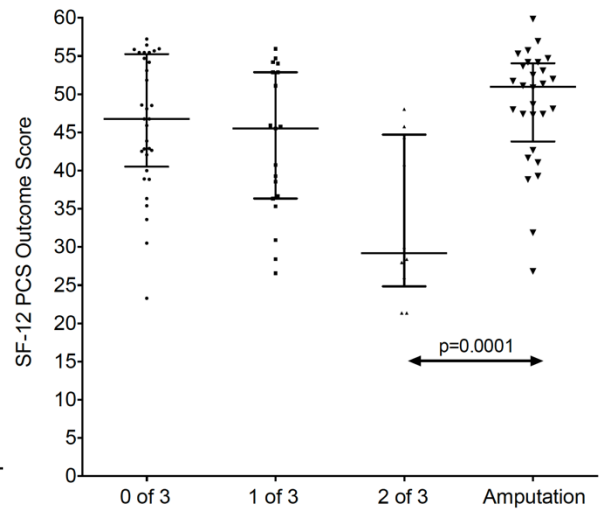


Figure 2.9. Graph showing the variation in SF-12 PCS scores for those with reconstructed hindfoot injuries depending on presence of key variables in initial injury, compared with those patients requiring delayed amputation following attempted reconstruction.

I found that the median SF-12 PCS of all 62 individuals retaining their limb was 45 (IQR 36-53), which was significantly lower than the 28 patients undergoing an amputation after initial salvage of their hindfoot injury ($p=0.0351$, Mann-Whitney), as shown in **Figure 2.8**. This poorer outcome is more pronounced when the injuries are grouped according to the presence of one or more of the three key variables previously identified, as shown in **Figure 2.9**.

2.4 Systematic review of preclinical therapies to prevent or treat fracture non-union

Appendix 6

Bennett, P., et al. (2018). "Preclinical therapies to prevent or treat fracture non-union: a systematic review." *PloS one* **13**(8): e0201077

As work progressed through the series of studies described I observed that rates of fracture non-union seemed higher than would be expected in equivalent civilian injuries (**Figure 2.10**).

Fracture non-union occurs when the normal healing processes of bone cease to the extent that solid healing cannot occur without further intervention³⁵. Wide variation on the incidence of non-union exists depending not only on anatomical location but on management and patient factors. Anything that disrupts a fracture's healing mechanism has the potential to cause non-union. In a review of 309,330 fractures in 18 bones³⁶ it was found that the odds ratio (OR) for developing non-union was significantly increased for certain risk factors including number of fractures (OR 2.65, 95% CI 2.34-2.99), open fractures (OR 1.66, 95%CI 1.55-1.77) and high energy injuries (OR 1.38, 95% CI 1.27-1.49).

Analysis of all 2,348 British military personnel who were injured and survived in Iraq and Afghanistan³⁷ showed that 886 (37.7%) had at least one fracture, with further detail provided in **Table 2.7**. A total of 1,530 fractures were recorded in this period, the majority of which (1,029/1,530 67%) involved the lower limb and pelvis. Of these, 597 (597/1,029 58%) were open fractures. Of all injuries sustained in this period, explosives were the commonest mechanism of injury (60%), with gunshot wounds accounting for an additional 23%.

Combat injuries have therefore been shown to be sustained through high-energy mechanisms which cause multiple open fractures, thereby explaining the perceived differences between rates of non-union seen in military and civilian patients.

Fracture non-union in military patients is associated with substantial morbidity. It was responsible for both of the delayed trans-femoral amputations I described in section 2.3.2, and for 43% of the delayed trans-tibial amputations described in section 2.3.3. It was also responsible for both early and delayed amputation following hindfoot fractures. As I have described in section 2.3.1 patients with more proximal amputations have poorer functional outcomes than those seen in patients with more distal amputations.

Although multiple therapeutic options exist that aim to prevent or treat fracture non-union, there is little conclusive evidence from high-quality studies as to the

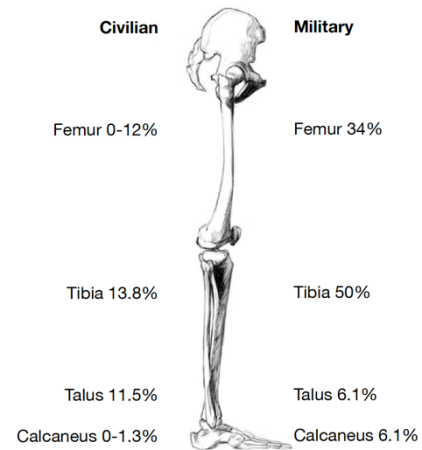


Figure 2.10. Schematic comparing rates of fracture non-union in military casualties to those seen in civilian series

Table 2.7. Fracture burden by individual (n=886)

Fractures	n(%)
Single fracture	530 (59.8)
Two fractures	208 (23.5)
Three or more fractures	148 (16.7)

efficacy of one particular treatment option over another. The realization that military patients are predisposed to fracture non-union and that treatment options are currently unsatisfactory led me to conduct the final study submitted as part of this PhD.

Preclinical studies are defined as those using animals to determine if a treatment is likely to be effective, before progression to testing in humans³⁸. When I was searching the literature in this field after identifying the higher rates of fracture non-union in military patients it was not clear on what basis researchers select potential therapies for translation into clinical studies from preclinical trials. It seemed to me that positive results from a single or small number of animal studies were used to justify progression to clinical trials, however problematic this seemed. There was certainly no evidence that researchers compared different preclinical studies in an attempt to determine which therapies were the most promising and therefore should be prioritized for translation into clinical trials.

Aims

Undertake systematic review to establish the range of therapies under investigation at the preclinical stage for the prevention or treatment of fracture non-union.

Methods

I searched MEDLINE and Embase from 1st January 2004 to 10th April 2017 for controlled trials evaluating an intervention to prevent or treat fracture non-union. Along with another reviewer acting independently I screened all titles and abstracts as per the inclusion and exclusion criteria (**Table 2.8** and **Figure 2.11**).

Results

After duplicates were removed I identified 5,171 records in the literature search. After I applied inclusion/exclusion criteria I was able to include 197 studies in the systematic review describing 204 different interventions (see **Figure 2.11**).

I found that substantial heterogeneity across studies in terms of type and site of defect, method of defect creation, species, length of follow-up and method of outcome reporting precluded pooling of study results. Despite the large amount of data it was not possible for me to make a valid comparison between any two studies, nor draw firm conclusions regarding the relative efficacies of different interventions and therefore identify those therapies that should be prioritized in translation research.

As such, despite the large number of preclinical trials investigating fracture non-union I identified, it is not possible to directly compare the efficacy of one therapy over another. It is therefore not possible to focus research efforts on a discrete number of therapies, nor justify the progression of the most promising to clinical trials.

Table 2.8: Summary of study inclusion and exclusion criteria

Inclusion Criteria	
Types of studies	Controlled trials
	Unpublished and published works
Types of participants	Mammalian model testing an intervention to treat or prevent fracture non-union
	Induced co-morbidities
Intervention	Interventions aim to: <ul style="list-style-type: none"> - Prevent non-union - Treat non-union - Promote or accelerate healing of a bony defect - Treat or ameliorate delayed union
	Administered after formation of a bony defect
	Established interventions in a novel vehicle
Comparator	Control group described receiving: <ul style="list-style-type: none"> - No treatment - Current standard of care - Alternative treatment
Outcome measures	Quantifiable measure of bone formation through radiological and/or histological means
Exclusion Criteria	
Types of studies	Review articles
Types of participants	Clinical trials
Intervention	Any intervention that has subsequently progressed to clinical trial

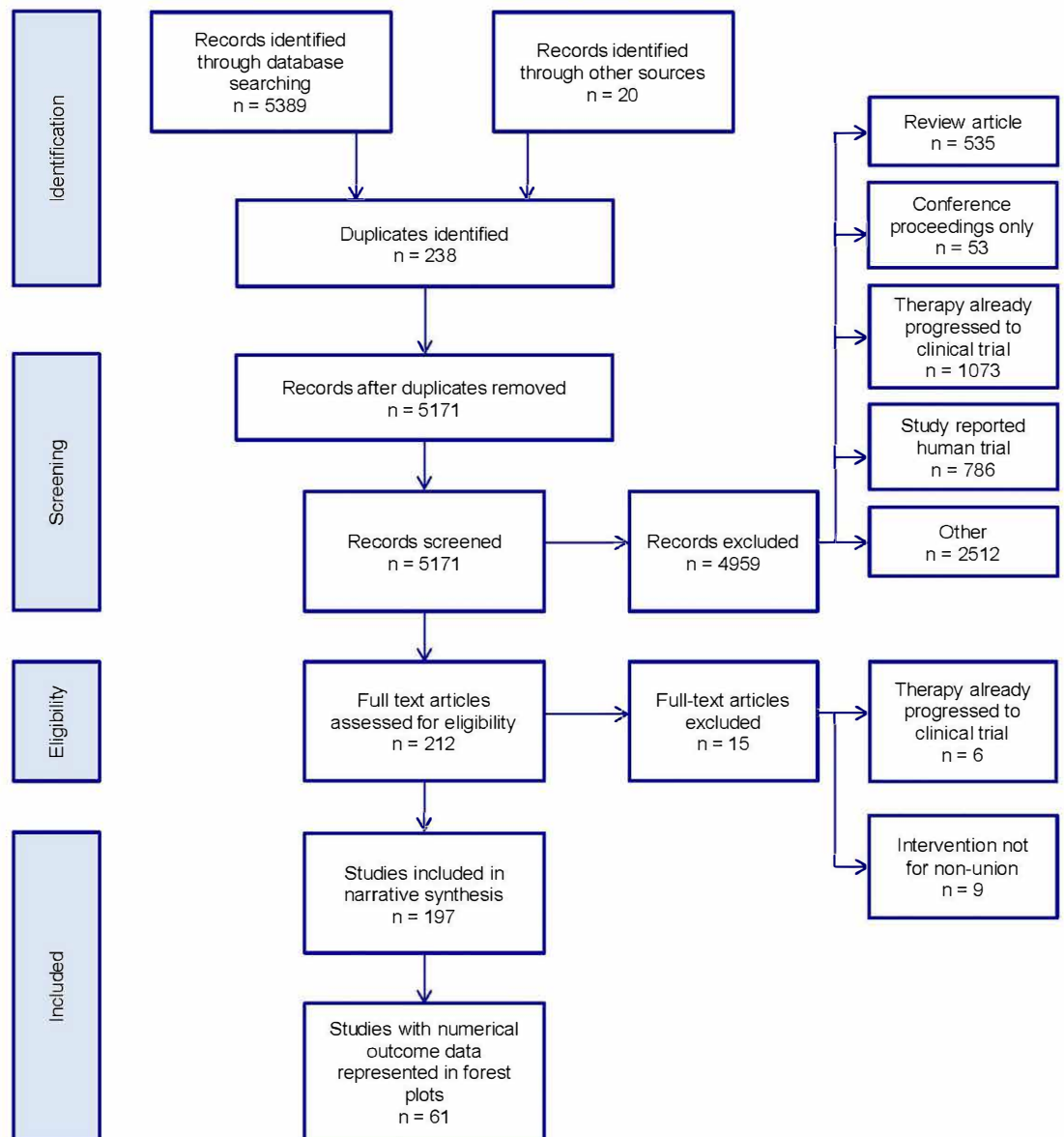


Figure 2.11. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISIMA) flow-diagram for exclusion of papers included in systematic review.

Recommendations

I commenced this review hoping to identify a discrete number of preclinical therapies aiming to prevent or treat fracture non-union that may be ready for translation to clinical trials. This would provide a focus for future military research to address the substantial burden of fracture non-union in military patients. Unfortunately due to the poor quality of contemporary animal research in this field this has not been realized.

To address the problems identified in this systematic review I believe the orthopaedic trauma community need to reach a global consensus on preferred animal models of bone healing. Similar consensus has been reached with the standardisation of fracture classification using the OTA/AO/Muller system³⁹. Once a consensus on the standardisation of species, defect and outcome measure is achieved, funding could be restricted to researchers using agreed models and methodology.

2.5 Summary

I have described five clinical papers examining the injuries and outcomes of British military personnel following severe lower limb trauma in combat during the Operations of Iraq and Afghanistan. I have also summarized a systematic review of preclinical therapies aiming to prevent or treat fracture non-union after I identified that military injuries were predisposed to this condition and had higher rates than those seen in equivalent civilian injuries.

I have shown that in patients with unilateral lower limb loss those with more proximal amputations had poorer functional outcomes than those with knee disarticulations or transtibial amputations.

Over the last century the ability of orthopaedic trauma surgeons to successfully salvage limbs following open fractures evolved from the challenges of the open

femur fracture during the First World War, to the open tibia fracture during the Vietnam War, to finally rest on the hindfoot injuries seen from Iraq and Afghanistan.

During combat Operations in Iraq and Afghanistan there were no fatalities following an isolated open femur fracture. However I have shown how segmental bone loss in these patients is associated with a poor outcome, and how non-union following femoral fracture is associated with a requirement for delayed amputation.

Similarly, although reconstruction is the mainstay of treatment for open tibia fractures the development of deep infection here is catastrophic and predisposes patients to poor outcomes and delayed amputation.

It is now with hindfoot fractures that orthopaedic surgeons face the challenge of addressing the difference between whether they *could* salvage the limb, and whether they *should*. Even in fractures that eventually unite and wounds that heal patients experience pain, and certain injury features I identified predispose patients to a poor outcome. Those patients in whom attempts at limb reconstruction fail and who subsequently require a delayed amputation have worse functional outcomes than those who undergo early amputation.

References

1. No authors listed. Allied Joint Doctrine for Medical Support. In: Ministry of Defence, editor. Edition B Version 1 ed: NATO Standardization Office; 2015.
2. Russell R, Bess A. JSP 999: Clinical Guidelines for Operations. *London: UK Ministry of Defence* 2012.
3. Hodgetts T, Mahoney P, Russell M, Byers M. ABC to< C> ABC: redefining the military trauma paradigm. British Association for Accident and Emergency Medicine; 2006.
4. Hodgetts T, Turner L, Grieves T, Paynes S. Major Trauma Effectiveness Project: report of Operation Agricola, Kosovo 1999. London: Defence Logistics Organisation, 2000.
5. Russell R, Hodgetts T, McLeod J, et al. The role of trauma scoring in developing trauma clinical governance in the Defence Medical Services. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2011; **366**(1562): 171-91.
6. Hodgetts T, Mahoney P, Russell R, Davies S, McLeod J. Trauma governance in the UK defence medical services. *Journal of the Royal Army Medical Corps* 2007; **153**(4): 239-42.
7. Mercer SJ, Kingston EV, Jones CP. The trauma call. *BMJ* 2018; **361**: k2272.
8. American Association for Automotive Medicine. Committee on Injury Scaling SJ. The abbreviated injury scale: AIS-80. 1980 revision. *American Association for Automotive Medicine* 1980.
9. Gennarelli TA, Wodzin E. AIS 2005: a contemporary injury scale. *Injury* 2006; **37**(12): 1083-91.
10. Penn-Barwell JG, Roberts SA, Midwinter MJ, Bishop JR. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003–2012. *Journal of Trauma and Acute Care Surgery* 2015; **78**(5): 1014-20.
11. Penn-Barwell JG, Fries CA, Bennett PM, Midwinter M, Baker AB. The injury burden of recent combat operations: mortality, morbidity, and return to service of U.K. naval service personnel following combat trauma. *Mil Med* 2013; **178**(11): 1222-6.
12. icasualties.org. Coalition Casualty Count. 2015.
<http://icasualties.org/OEF/Nationality.aspx?hndQry=UK> (accessed 6 January 2016).
13. No authors listed. The Abbreviated Injury Scale 2005: Military.: Des Plaines: Association for the Advancement of Automotive Medicine; 2005.
14. Baker SP, o'Neill B, Haddon Jr W, Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *Journal of Trauma and Acute Care Surgery* 1974; **14**(3): 187-96.
15. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *Journal of Trauma and Acute Care Surgery* 1997; **43**(6): 922-6.
16. Champion HR, Holcomb JB, Lawnick MM, et al. Improved characterization of combat injury. *Journal of Trauma and Acute Care Surgery* 2010; **68**(5): 1139-50.
17. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Medical care* 1992.
18. Hays RD, Hahn H, Marshall G. Use of the SF-36 and other health-related quality of life measures to assess persons with disabilities. *Archives of physical medicine and rehabilitation* 2002; **83**: S4-S9.
19. Rommers GM, Vos LD, Groothoff JW, Eisma WH. Mobility of people with lower limb amputations: scales and questionnaires: a review. *Clinical rehabilitation* 2001; **15**(1): 92-102.
20. Jenkinson C, Layte R. Development and testing of the UK SF-12. *Journal of Health Services Research* 1997; **2**(1): 14-8.
21. Jenkinson C, Layte R, Jenkinson D, et al. A shorter form health survey: can the SF-12 replicate results from the SF-36 in longitudinal studies? *Journal of Public Health* 1997; **19**(2): 179-86.
22. Johanson NA, Liang MH, Daltroy L, Rudicel S, Richmond J. American Academy of Orthopaedic Surgeons lower limb outcomes assessment instruments. *J Bone Joint Surg Am* 2004; **86**(5): 902-9.
23. Tran T, Thordarson D. Functional outcome of multiply injured patients with associated foot injury. *Foot & ankle international* 2002; **23**(4): 340-3.
24. Soto-Hall R, Horowitz T. The treatment of compound fractures of the femur. *Journal of the American Medical Association* 1946; **130**(3): 128-34.

25. Miric D, Bumbasirevic M, Senohradski K, Djordjevic Z. Pelvifemoral external fixation for the treatment of open fractures of the proximal femur caused by firearms. *Acta orthopaedica belgica* 2002; **68**(1): 37-41.
26. Mack AW, Freedman BA, Groth AT, Kirk KL, Keeling JJ, Andersen RC. Treatment of open proximal femoral fractures sustained in combat. *JBS* 2013; **95**(3): e13.
27. Müller ME NS, Koch P,. Classification AO des fractures. Berlin: Springer-Verlag; 1987.
28. Nanchahal J, Nayagam S, Khan U, et al. Standards for the management of open fractures of the lower limb. London: Royal Society of Medicine Press Ltd; 2009.
29. Patzakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. *Clinical orthopaedics and related research* 1989; (243): 36-40.
30. Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. *J Bone Joint Surg (Am)* 1976; **58**(4): 453-8.
31. Dickens CPJF, Kilcoyne CKG, Kluk CMW, Gordon LCWT, Shawen LSB, Potter MBK. Risk factors for infection and amputation following open, combat-related calcaneal fractures. *The Journal of Bone & Joint Surgery* 2013; **95**(5): e24.
32. Griffin D, Parsons N, Shaw E, et al. Operative versus non-operative treatment for closed, displaced, intra-articular fractures of the calcaneus: randomised controlled trial. *British Medical Journal* 2014; **349**.
33. Cheung GC, Kumar G. The BMJ's cover line on calcaneal fractures is misleading. *BMJ* 2014; **349**.
34. Ramasamy MA, Hill CAM, Masouros S, et al. Outcomes of IED Foot and Ankle Blast Injuries. *The Journal of Bone & Joint Surgery* 2013; **95**(5): e25.
35. Brinker M. Nonunions: Evaluation and Treatment. In: Browner BD, Jupiter JB, Levine AM, Trafton PG, eds. *Skeletal Trauma Basic Science, Management, and Reconstruction*. Sixth ed. Philadelphia: Saunders; 2003.
36. Zura R, Xiong Z, Einhorn T, et al. Epidemiology of fracture nonunion in 18 human bones. *JAMA surgery* 2016; **151**(11): e162775-e.
37. Chandler H, MacLeod K, Penn-Barwell JG, et al. Extremity injuries sustained by the UK military in the Iraq and Afghanistan conflicts: 2003–2014. *Injury* 2017; **48**(7): 1439-43.
38. National Cancer Institute. NCI Dictionary of Cancer Terms.
<https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=445172017>.
39. Marsh J, Slongo TF, Agel J, et al. Fracture and dislocation classification compendium-2007: Orthopaedic Trauma Association classification, database and outcomes committee. LWW; 2007.

SECTION 3

CONTRIBUTION TO ORIGINAL KNOWLEDGE

3.0 Introduction

I have presented six papers which constitute this PhD submission. In the first five papers I described the injuries and outcomes of British military personnel following severe lower limb trauma sustained during combat Operations in Iraq and Afghanistan. I presented a systematic review of preclinical therapies aiming to prevent or treat non-union. Fracture non-union therefore remains a common clinical problem of particular relevance to the military and without a definitive solution.

3.1 Clinical Studies

3.1.1 Unilateral lower limb amputation

In this paper I presented the functional outcomes for patients with a unilateral lower limb amputation. By using follow-up data I showed for the first time that patients with more proximal amputations have worse functional outcome scores than those with more distal amputations. Although SF-36 outcomes have previously been described in military patients with unilateral lower limb loss they have not distinguished between amputation levels¹⁻³.

This paper was unable to resolve the question of amputation through the knee, as I did not show any statistical difference when comparing functional outcomes of those with knee disarticulations to transfemoral amputees. Historically knee disarticulations have been avoided as they were shown to be associated with a lower quality of life and more pain than those with transfemoral amputations⁴. More recently this has been challenged by the findings of a meta-analysis of patients undergoing amputation for trauma⁵. This remains a contentious issue where knee disarticulation is not currently recommended by the British Association of Plastic, Reconstructive and Aesthetic Surgeons/British Orthopaedic Association (BAPRAS/BOA) standards for managing open lower limb fractures⁶.

I also described how the British military's approach to managing limb amputation evolved throughout the conflicts in Iraq and Afghanistan. This was the first time this strategy had been presented in the literature. In previous wars amputation was performed proximal to the zone of injury. This allowed for the formation of a robust stump with healthy wound edges⁷, typically requiring only two surgical procedures¹.

During Iraq and Afghanistan the surgical strategy focused on maximizing the length of the residual limb. This was achieved with serial debridements over several operations, where only non-viable tissue from within the zone of injury was excised. Plastic surgical techniques were used to ensure closure of the soft tissue envelope over the bone. This approach resulted in a longer residual limb, a strategy which is supported by my findings that patients with longer limbs have better functional outcomes.

3.1.2 Open femur fractures

Open femur fractures are relatively uncommon injuries in the civilian setting typically seen in polytrauma patients following road traffic collisions⁸. Series in the literature tend to focus on the advantages and disadvantages of one fixation technique over another⁹, or the timing of surgery¹⁰⁻¹³.

In my paper I described the entire cohort of British military patients with open fractures of the femur sustained during combat Operations in Iraq and Afghanistan. With no recorded fatalities following an isolated open femur fracture I used the rates of fracture union at 12-months as an indicator of the adequacy of treatment.

For the first time in the literature I was able to show with this cohort of patients the significance of segmental bone loss on the rates of fracture union and subsequent requirement for transfemoral amputation. I presented the importance of the level of amputation on eventual functional outcome in the previous paper. This study

therefore emphasizes the significance bone loss in the context of an open femur fracture may have on an individual's eventual recovery.

3.1.3 Open tibia fractures

The importance and challenges of avoiding deep infection in open tibial fractures is well recognized by the orthopaedic community⁶. In this study I presented the cohort of open tibia fractures from Iraq and Afghanistan and described how only half had achieved fracture union 12-months after injury. I showed that deep infection in these patients was associated with a significant requirement for revision surgery, with a subset subsequently requiring amputation. Conversely, the results I presented did not show any association between the method of fixation, degree of bone loss or requirement for vascularized soft tissue coverage on subsequent fracture union.

In the editorial of the journal this work was published in, the study was described as, "a real and genuine addition to orthopaedic excellence [...] first class research [...] the importance of the aggressive prevention of infection"¹⁴.

3.1.4 Hindfoot fractures

In the first of the pair of hindfoot papers I identified the cohort of patients sustaining hindfoot fractures during combat Operations in Iraq and Afghanistan, then presented the medium-term functional outcomes in the second study. These papers, along with an editorial discussion¹⁵, introduce the concept of *could* versus *should* when considering limb reconstruction in these patients.

In this analysis I demonstrated that in the short-term deep infection was associated with a requirement for limb amputation at 18-months. I then presented the medium-term functional outcomes and showed that those patients subsequently requiring trans-tibial amputation after attempted limb reconstruction efforts had failed had improved functional outcome scores when compared to those with

“successful” limb salvage, i.e. those who had retained their limbs. I also identified three injury variables that could be identified at the time of wounding that were associated with a significantly poorer functional outcome than those injuries without these variables.

Taking the results of these papers together for the first time I am able to provide surgeons with evidence to help counsel patients about their likely recovery when considering limb reconstruction over amputation. I emphasise the difference between success from a surgeon’s perspective and patient’s perspective and that even achieving fracture union and healed wounds does not necessarily provide patients with an acceptable quality of life.

3.2 Systematic review of preclinical therapies to prevent or treat fracture non-union

In the clinical papers examining combat fractures of the femur, tibia and hindfoot I identified higher than expected rates of fracture non-union. This is of particular concern to military surgeons as their patients typically present with multiple fractures which are often open and sustained through high-energy mechanisms, predisposing them to this condition. There is no single, accepted treatment for the effective prevention or treatment of fracture non-union. As such I undertook a systematic review of the preclinical literature in an attempt to identify therapies that showed promise for translational work into clinical trials.

There have been no previous systematic reviews of preclinical studies aiming to prevent or treat fracture non-union. As the methodology for systematic reviews of preclinical research is still evolving this study provided a valuable contribution to the existing evidence base. I was able to demonstrate a wide breadth of therapies currently under investigation at the preclinical phase for the prevention or treatment of fracture non-union. Unfortunately, rather than identify promising therapies for translational research through this review I instead demonstrated the

heterogenous nature of preclinical non-union research, and the problems with progressing therapies to clinical trials when no direct comparison of efficacy is possible due to significant methodological variances.

Through this systematic review I am however able to provide a foundation from which the global orthopaedic community can base a requirement to standardize preclinical non-union research in order to maximise the future benefit of ongoing work in this area.

References

1. Dougherty PJ. Transtibial amputees from the Vietnam War. *The Journal of Bone & Joint Surgery* 2001; **83**(3): 383-.
2. Gunawardena NS, de Alwis Seneviratne R, Athauda T. Functional outcomes of unilateral lower limb amputee soldiers in two districts of Sri Lanka. *Military medicine* 2006; **171**(4): 283.
3. Taghipour H, Moharamzad Y, Mafi AR, et al. Quality of life among veterans with war-related unilateral lower extremity amputation: a long-term survey in a prosthesis center in Iran. *Journal of orthopaedic trauma* 2009; **23**(7): 525-30.
4. MacKenzie EJ, Bosse MJ, Castillo RC, et al. Functional outcomes following trauma-related lower-extremity amputation. *The Journal of Bone & Joint Surgery* 2004; **86**(8): 1636-45.
5. Penn-Barwell JG. Outcomes in lower limb amputation following trauma: a systematic review and meta-analysis. *Injury* 2011; **42**(12): 1474-9.
6. Nanchahal J, Nayagam S, Khan U, et al. Standards for the management of open fractures of the lower limb. London: Royal Society of Medicine Press Ltd; 2009.
7. Coupland RM. Amputation for antipersonnel mine injuries of the leg: preservation of the tibial stump using a medial gastrocnemius myoplasty. *Annals of the Royal College of Surgeons of England* 1989; **71**(6): 405.
8. Court-Brown C, Rimmer S, Prakash U, McQueen M. The epidemiology of open long bone fractures. *Injury* 1998; **29**(7): 529-34.
9. Williams MM, Askins V, Hinkes EW, Zych GA. Primary reamed intramedullary nailing of open femoral shaft fractures. *Clinical orthopaedics and related research* 1995; (318): 182-90.
10. Lhowe DW, Hansen S. Immediate nailing of open fractures of the femoral shaft. *The Journal of bone and joint surgery American volume* 1988; **70**(6): 812-20.
11. Bone L, Johnson K, Weigelt J, Scheinberg R. Early versus delayed stabilization of femoral fractures. A prospective. *J Bone Joint Surg Am* 1989; **71**: 336-40.
12. Chapman MW, Mahoney M. The role of early internal fixation in the management of open fractures. *Clinical orthopaedics and related research* 1979; (138): 120-31.
13. Grosse A, Christie J, Taglang G, Court-Brown C, McQueen M. Open adult femoral shaft fracture treated by early intramedullary nailing. *The Journal of bone and joint surgery British volume* 1993; **75**(4): 562-5.
14. Villar R. Gatekeepers to quality. *Bone Joint Journal* 2013; **95-B**(1): 1.
15. Penn-Barwell JG, Bennett PM, Gray AC. The 'could' and the 'should' of reconstructing severe hind-foot injuries. Elsevier; 2017.

SECTION 4

METHODOLOGICAL CRITIQUE AND REFLECTION

4.0 Introduction

The collective papers submitted for this PhD were not conceived as a single body of work. Rather they represent a series of studies over eight years which followed a single thread of inquiry, and which developed in methodological sophistication over this period.

The gold standard of medical research, the Randomised Controlled Trial (RCT), has only been attempted once by either the UK or US military medical communities in the recent conflict, with limited success¹. Instead, the methodologies used in my studies were a pragmatic approach to the population of combat casualties with heterogenous injuries treated over a decade of conflict. The aims of the papers and therefore the research as a whole evolved as we obtained our results and the narrative I have described in previous chapters became clear. My understanding of how research should be conceived and conducted developed alongside this work. In this chapter I will present the training I have done in research methodology and discuss the limitations of the studies included.

4.1 Research training

Since starting the research submitted for this PhD I have undergone the following training in research methodology:

- Introduction to Good Clinical Practice (Secondary Care)
 - National Institute for Health Research eLearning, September 2015
- Research in Medical Education 20 credit module
 - University of Dundee, July 2019
- Research Methods in Science
 - University of Warwick eLearning, July 2019
- Research Methods in Literature review
 - University of Warwick eLearning, July 2019
- Research Integrity
 - Oxford University Press / Epigeum eLearning, July 2019

4.2 Designing research

Research starts with the formulation of a research question. With strong foundations in a thorough literature review the research question should be specific, focused and clearly communicate what the research will attempt to answer.

In the gold-standard RCT the 'PICO' approach (population, intervention, comparator, outcome) provides a framework for study design. However before the PICO approach can be used to design prospective studies, observational research is necessary to identify and define problems, and refine research questions and hypotheses. For example, an RCT to test an intervention believed to reduce infection after open fractures would first need to determine that infection after combat open fractures was a frequent complication with clinically relevant complications, as well as the rate of infection and how this could be defined in a specific population. Although observational studies often provide the literature that research questions for RCTs are founded on, they are also used to evaluate associations between variables and outcomes, and have a separate utility when ethical or feasibility concerns limit the use of a RCT².

My observational studies included in this PhD define and quantify the clinical problems from both the perspective of the treating surgeons and the patients following over a decade of conflict in Iraq and Afghanistan. Not since the Second World War have British military surgeons been challenged by the scale of combat casualties as sustained in the last fifteen years. As such there is a complete absence of contemporary literature describing modern medical approaches to large numbers of battlefield casualties. My work therefore set out to define the British military's experiences and provide a foundation for conducting future prospective studies.

Furthermore having identified and quantified the problem of bony non-union following combat fracture I sought to systematically determine the most effective

emerging treatments which could be selected for translational studies as the basis for RCTs using a PICO framework.

4.3 Methodological weaknesses

4.3.1 Registries

Registry searches are a common way to generate large datasets for observational studies. In very large datasets, for example the National Joint Registry (NJR), the vast numbers are assumed to limit the systematic biases of incomplete or poor-quality data. My studies were based on searches of the comparatively small Joint Theatre Trauma Registry (JTTR) and therefore my data was more likely to be affected by incomplete entries or poor-quality coding. In all studies I sought to mitigate this by broadening the search categories to use all related codes and then individually checking clinical records. For example, when identifying patients with open diaphyseal tibial fractures I searched for codes that included bony injuries of the knee, tibia or ankle and confirmed the exact anatomical location by reviewing radiographs to identify those that met the study inclusion criteria. This aimed to obtain the highest possible quality dataset for the basis of this research.

4.3.2 The use of controls

Pre-determined control groups, where the researchers alter variables that subjects are exposed to, do not form part of observational study methodology². Instead, disease status is characterised and potential risk factors are then examined in those with and without the disease in case-control observational studies³. My studies group patients according to their injury, treatment or outcome characteristics. For example, in the cohort of patients with open tibial fractures I wanted to identify risk factors for requiring revision surgery and found an association with deep infection. In this way observational studies allow association rather than causation to be ascribed, and should consider other explanations for their findings⁴.

4.3.3 Follow-up

Sufficient follow-up numbers are a frequent problem in clinical studies, with no consensus on acceptable “loss to follow-up” rates⁵. The longer the duration of follow-up the more attrition of follow-up inevitably occurs. In my initial studies that were focused on surgically defined outcomes i.e. infection, bone union, amputation, I accepted a lower minimum duration of follow-up of 12 months, in order to minimise the loss to follow-up rate. For example in the first published study from this body of work follow-up of 91% at 12 months was achieved⁶. However for studies designed to measure patient reported outcomes after these complex injuries I recognised that recovery evolves over many years and therefore an ambitious minimum follow-up period of 5 years was used, whilst accepting this would increase the loss to follow-up rate. Although a 5 year follow-up rate of 67% was obtained in the cohort of patients with hindfoot fractures⁷, this is not dissimilar to large observational studies in civilian settings. For example in the Lower Extremity Assessment Project, frequently viewed as the benchmark of follow-up in trauma patients, a follow-up rate of 77% at 24 months was obtained⁸.

4.3.4 Outcomes

My studies use both surgeon- and patient-reported outcome measures. Initial studies were focused on answering questions most immediately relevant to clinicians treating these casualties, for example deep infection, amputation, revision and non-union. However I recognise that outcomes significant to clinicians might not be the most important to patients. Therefore in later studies conducted at a time where longer follow-up was possible I shifted my focus to outcome measures more relevant to patients’ experience of their own recovery.

The challenge in selecting the most appropriate outcome measure to use is the balance between the ability to generalise results across different injuries versus the precise measurement of injury specific outcomes. For example, when attempting to compare outcomes between those undergoing lower limb amputation or limb salvage surgery for hindfoot fractures, solely using the American Academy of

Orthopaedic Surgeons' Foot and Ankle (AAOS F&A) outcome questionnaire would not have allowed us to compare function of those retaining their limb to those subsequently requiring amputation. The AAOS F&A score did however provide a more accurate representation of function following hindfoot injury, rather than overall function determined by general outcome scores.

At the time of conducting our study of lower limb amputees, no specific outcome measures for combat casualties existed. After observing that US servicemen were achieving maximal scores on standard outcome measures shortly after being fitted with their initial prosthesis, military clinicians developed and validated a tool specifically for male servicemen with traumatic lower limb loss⁹. As this tool was not available at the time of our work all of our PROM studies used the SF-12 or SF-36 tools for general outcome measures. This had the advantage of allowing comparison with similar studies either historically¹⁰ or those published later, for example, the Military Extremity Trauma Amputation/Limb Salvage study¹¹. Similar to the US clinicians assessing outcome after lower limb amputation, we also encountered limitations of using civilian scoring systems in our military population of patients. There seemed to be a ceiling effect with the AAOS F&A score when assessing recovery in patients with hindfoot fractures. This is likely due to differences in populations studied, as the tool was originally developed and validated in patients with osteoarthritis rather than trauma. The obvious disadvantage of using general outcome scores such as SF-12 and SF-36 in our patient population will be when our cohort may be influenced by other injuries such as upper limb loss significantly affecting quality of life.

4.4 Generalisability

The cohorts of patients in all five clinical papers are British military casualties, predominantly injured through blast and ballistic mechanisms. The generalisability of these findings to the wider civilian population must therefore be considered carefully and caution exercised before extrapolating them too widely. However

terrorist incidents are increasing worldwide¹² and it is therefore not inconceivable that civilian surgeons will need to treat victims injured by blast and ballistics¹³. Lessons learnt by British military surgeons and presented in these clinical papers may need to be applied by civilian surgeons facing similar injuries.

Notwithstanding mechanisms of injury, military and civilian patients differ in other ways. Deployed military personnel tend to be younger, free of comorbidities and with a high level of pre-injury fitness. Many of them expect to return to the same high level of function they had prior to their injury¹⁴. Military casualties benefit from a very clear treatment pathway with multiple specialists and members of the multi-disciplinary team involved in their care from their beginning. Though the benefits of this model are increasingly recognised and applied to civilian trauma care¹⁵ the rehabilitation pathway and resources available to military patients are more clearly defined and better funded resulting in improved outcomes¹⁶.

4.5 Future research

Since the cessation of combat operations in Iraq and Afghanistan significant changes have been made to the management of patients both with lower limb amputation and retained limbs.

Osseointegration (OI) describes a surgical procedure where an external prosthesis is directly attached to the skeleton through the use of an intramedullary device, negating the requirement for sockets¹⁷. This technique is of particular benefit to patients with high trans-femoral amputation (TFA) where problems with socket fitting may prevent ambulation. It may be that patients who have OI prosthesis of their TFA will now report improved quality of life, and close follow-up of this cohort will be required.

For patients with blast injured hindfeet the use of offloading braces particularly in US military patients is showing potential¹⁸, and their use in British military casualties is likely to be of particular interest. Thus for both amputation and limb

reconstruction patients new surgical and rehabilitative options are changing the way injuries may be managed in the future, and will once again shift the paradigm of the *should versus could* debate of salvage over amputation.

Finally, the studies I have presented for this PhD define surgeon reported outcomes after lower limb trauma alongside patient reported outcome measures. Having identified fracture non-union as being a particular problem in military patients the systematic review examining preclinical therapies for fracture non-union failed to identify a single therapy for translational research. Future work should focus on developing and validating a standardised model of non-union to be adopted by organisations such as AO and Orthopaedic Trauma Association (OTA). Once effective therapies for non-union are established at the pre-clinical phase, translational research can begin in humans, which would then provide the foundation for a RCT using the PICO approach. Researchers should also investigate why military patients are at higher risk of fracture non-union with consideration given to the effects of ballistic wounding on osteology and cell function.

References

1. Fries C, Ayalew Y, Penn-Barwell J, Porter K, Jeffery S, Midwinter M. Prospective randomised controlled trial of nanocrystalline silver dressing versus plain gauze as the initial post-debridement management of military wounds on wound microbiology and healing. *Injury* 2014; **45**(7): 1111-6.
2. Johnson LL. Design of Observational Studies. In: Gallin JI, Ognibene FP, Johnson LL, eds. *Principles and Practice of Clinical Research*. Fourth ed. London, United Kingdom: Elsevier Academic Press; 2018: 231-48.
3. Newman TB, Browner WS, Cummings SR, Hulley SR. Designing Case-Control Studies. In: Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB, eds. *Designing Clinical Research*. Fourth ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2013.
4. Newman TB, Browner WS, Hulley SB. Enhancing Causal Inference in Observational Studies. In: Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB, eds. *Designing Clinical Research*. Philadelphia, USA: Lippincott Williams & Wilkins; 2013.
5. Zelle BA, Bhandari M, Sanchez AI, Probst C, Pape H-C. Loss of follow-up in orthopaedic trauma: is 80% follow-up still acceptable? *Journal of Orthopaedic Trauma* 2013; **27**(3): 177-81.
6. Penn-Barwell JG, Bennett PM, Fries CA, Kendrew JM, Midwinter MJ, Rickard RF. Severe open tibial fractures in combat trauma: management and preliminary outcomes. *Bone Joint J* 2013; **95-B**(1): 101-5.
7. Bennett P, Stevenson T, Sargeant I, Mountain A, Penn-Barwell J. Outcomes following limb salvage after combat hindfoot injury are inferior to delayed amputation at five years. *Bone and Joint Research* 2018; **7**(2): 131-8.
8. MacKenzie EJ, Bosse MJ, Castillo RC, et al. Functional outcomes following trauma-related lower-extremity amputation. *The Journal of Bone & Joint Surgery* 2004; **86**(8): 1636-45.
9. Gailey RS, Gaunaud IA, Raya MA, et al. Development and reliability testing of the Comprehensive High-Level Activity Mobility Predictor (CHAMP) in male servicemembers with traumatic lower-limb loss. *Journal of Rehabilitation Research and Development* 2013; **50**(7): 905-18.
10. Penn-Barwell JG. Outcomes in lower limb amputation following trauma: a systematic review and meta-analysis. *Injury* 2011; **42**(12): 1474-9.
11. Frisch HM, Andersen CRC, Mazurek CMT, et al. The military extremity trauma amputation/limb salvage (METALS) study. *The Journal of Bone & Joint Surgery* 2013; **95**(2): 138-45.
12. Edwards D, McMenemy L, Stapley S, Patel H, Clasper J. 40 years of terrorist bombings—a meta-analysis of the casualty and injury profile. *Injury* 2016; **47**(3): 646-52.
13. Wilson N, d'Ardenne P, Brewin CR, Catchpole M. How Can the Experience of a Terrorist Attack Inform Public Health Priorities? Some Lessons from the London 7/7 Bombings. *International Perspectives on Terrorist Victimisation*: Springer; 2015: 149-69.
14. Penn-Barwell JG, Fries CA, Bennett PM, Midwinter M, Baker AB. The injury burden of recent combat operations: mortality, morbidity, and return to service of U.K. naval service personnel following combat trauma. *Mil Med* 2013; **178**(11): 1222-6.
15. British Orthopaedic Association. BOAST 13: Rehabilitation and communication with trauma patients. <https://www.boa.ac.uk/standards-guidance/boasts.html>: British Orthopaedic Association; 2016.
16. Etherington J, Bennett AN, Phillip R, Mistlin A. Outcomes for UK service personnel indicate high quality trauma care and rehabilitation. *BMJ: British Medical Journal (Online)* 2016; **354**.
17. Al Muderis M, Lu W, Li JJ. Osseointegrated Prosthetic Limb for the treatment of lower limb amputations. *Der Unfallchirurg* 2017; **120**(4): 306-11.
18. Highsmith MJ, Nelson LM, Carbone NT, et al. Outcomes associated with the Intrepid Dynamic Exoskeletal Orthosis (IDEO): a systematic review of the literature. *Military medicine* 2016; **181**(suppl_4): 69-76.

CONFIRMATION OF CONTRIBUTION LETTERS



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 2019

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering this work. She conducted data collection and data analysis in addition to preparing the manuscript.

1. Preclinical therapies to prevent or treat fracture non-union: a systematic review

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

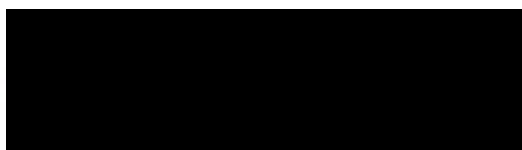
Yours aye,

A handwritten signature in black ink, appearing to read "D Bem".

Dr D Bem PhD
Systematic reviewer
Institute of Applied Health Research



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 2019

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering this work. She conducted data collection and data analysis in addition to preparing the manuscript.

1. Preclinical therapies to prevent or treat fracture non-union: a systematic review

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

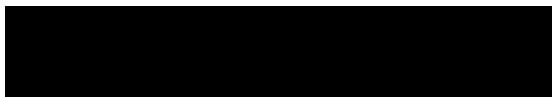
Yours aye,

16th May 2019.

Ms J Dretzke MSc
Senior systematic reviewer
Institute of Applied Health Research



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



21 May 2019

To whom it may concern

As one of the co-authors of the below paper I can confirm that Miss Philippa Bennett played a pivotal role in delivering this work. She conducted data collection, data analysis and the majority of the manuscript preparation.

1. Severe open tibial fractures in combat trauma: management and preliminary outcomes

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Surg Cdr CA Fries MSc FRCS
Consultant Plastic Surgeon
John Radcliffe Hospitals



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 2019

To whom it may concern

As one of the co-authors of the below paper I can confirm that Miss Philippa Bennett played a pivotal role in delivering this work. She conducted data collection, data analysis and the majority of the manuscript preparation.

1. Severe open tibial fractures in combat trauma: management and preliminary outcomes

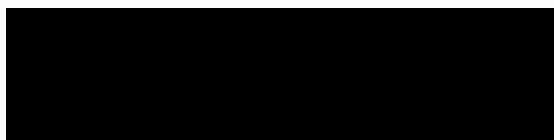
This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Gp Capt J Kendrew FRCS (Tr & Orth)
Consultant Trauma & Orthopaedic Surgeon
Royal Centre for Defence Medicine



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 2019

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering these works. She was instrumental in the design of the studies, in addition to conducting data collection and analysis and manuscript preparation.

1. Outcomes following limb salvage after combat injury are inferior to delayed amputation at five years
2. Preclinical therapies to prevent or treat fracture non-union: a systematic review

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Lt Col A Mountain FRCS (Tr & Orth)
Consultant Trauma & Orthopaedic Surgeon
University Hospital of North Midlands



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 Mar 2019

To whom it may concern

As one of the co-authors of the below paper I can confirm that Miss Philippa Bennett played a pivotal role in delivering this work. She conducted data collection, data analysis and the majority of the manuscript preparation.

1. The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Surge Lt Cdr RW Myatt MRCS
Specialist Registrar, Trauma & Orthopaedics
Institute for Naval Medicine



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



[REDACTED]
[REDACTED]@m

10 May 2019

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering these works. She conducted data collection, data analysis and the majority of the manuscript preparation for all of them, and was instrumental in the design of the two hindfoot studies.

1. Unilateral lower limb loss following combat injury: medium-term outcomes in British military amputees
2. Severe open tibial fractures in combat trauma: management and preliminary outcomes
3. The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period
4. Salvage of combat hindfoot fractures in 2003-2014 UK military
5. Outcomes following limb salvage after combat injury are inferior to delayed amputation at five years
6. Preclinical therapies to prevent or treat fracture non-union: a systematic review

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Surg Cdr JG Penn-Barwell MSc FRCS (Tr & Orth)
Consultant Orthopaedic Trauma Surgeon
John Radcliffe Hospitals



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 2019

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering these works. She conducted data collection, data analysis and the majority of the manuscript preparation for all of them, and was instrumental in the design of the two hindfoot studies.

1. Unilateral lower limb loss following combat injury: medium-term outcomes in British military amputees
2. The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period
3. Salvage of combat hindfoot fractures in 2003-2014 UK military
4. Outcomes following limb salvage after combat injury are inferior to delayed amputation at five years

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Gp Capt ID Sargeant OBE FRCS (Tr & Orth)
Consultant Orthopaedic Trauma Surgeon
Royal Centre for Defence Medicine



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 201

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering these works. She was instrumental in the design of the studies, in addition to conducting data collection and analysis and manuscript preparation.

1. Outcomes following limb salvage after combat injury are inferior to delayed amputation at five years
2. Preclinical therapies to prevent or treat fracture non-union: a systematic review

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

Surg Lt Cdr T Stevenson MRCS
Specialist Registrar, Trauma & Orthopaedics
Institute of Naval Medicine



Institute of Naval Medicine
Crescent Road
ALVERSTOKE
Hampshire
PO12 2DL



10 May 2011

To whom it may concern

As one of the co-authors of the below papers I can confirm that Miss Philippa Bennett played a pivotal role in delivering this work. She conducted data collection and data analysis in addition to preparing the manuscript.

1. Preclinical therapies to prevent or treat fracture non-union: a systematic review

This research was conducted through the Severe Lower Extremity Combat Trauma (SeLECT) study group. This collaborative was co-founded by Miss Bennett as an undergraduate medical student while working at the Royal Centre for Defence Medicine in Birmingham.

Yours aye,

A handwritten signature in black ink, appearing to be "SK Stewart".

Maj SK Stewart MRCS
Specialist Registrar, Trauma & Orthopaedics
Royal Centre for Defence Medicine

BIBLIOGRAPHY

1. Book chapters

Penn-Barwell J, Bennett PM, Heil K. Firearms, ballistics and gunshot wounds. In: Greaves I, Porter K, Wright C, editors. *Trauma Care Pre-Hospital Manual*. Boca Raton, FL: Taylor & Francis Group; 2019. p. 243-55

Penn-Barwell J, Bennett PM, Heil K. Blast injuries. In: Greaves I, Porter K, Wright C, editors. *Trauma Care Pre-Hospital Manual*. Boca Raton, FL: Taylor & Francis Group; 2019. p. 256-63

2. Published articles

Bennett PM., Stewart, S. K., Dretzke, J., Bem, D., & Penn-Barwell, J. G. (2018). Preclinical therapies to prevent or treat fracture non-union: A systematic review. *PloS one*, 13(8), e0201077.

Penn-Barwell, J. G., Bennett PM., & Gray, A. C. (2018). The 'could' and the 'should' of reconstructing severe hind-foot injuries. *Injury*, 49(2), 147-148.

Bennett PM, Stevenson T, Sargeant ID, Mountain A, Penn-Barwell JG. Outcomes following limb salvage after combat hindfoot injury are inferior to delayed amputation at five years. *Bone and Joint Research* 2018;7-2:131-8.

Bennett PM, Stevenson T, Sargeant ID, Mountain A, Penn-Barwell JG. Salvage of Combat Hindfoot Fractures in 2003-2014 UK Military. *Foot & Ankle International* 2017;38(7): 745-751.

Penn-Barwell JG, Bennett PM, Mortiboy DE, Fries CA, Groom AFG, Sargeant ID. Factors influencing infection in 10-years of battlefield open tibia fractures. *Strat Trauma Limb Recon*. 2016 11(1), 13-18

Stewart SK, Bennett PM, Stapley SA, Dretzke J, Bem, D, Penn-Barwell JG. Pre-clinical evaluation of therapies to prevent or treat bone non-union: a systematic review protocol. *Systematic Reviews* 2015 12;4(1):161

McMenemy L, Bennett PM, Stapley SA. The Ship's Doctor in the Royal Navy – 100 years of tradition and progress. *J R Nav Med Serv* 2015;101(1):15-19

Bennett PM, Sargeant ID, Myatt RW, Penn-Barwell JG. The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period. *Bone Joint J* 2015;97(6):842-846

Penn-Barwell JG, Myatt RW, Bennett PM, Sargeant ID. Medium-term outcomes following limb salvage for severe open tibia fractures are similar to trans-tibial amputation. *Injury* 2015;46(2):288-91

- Penn-Barwell JG, Fries CA, Bennett PM, Midwinter MJ, Baker A. Mortality, survival and residual injury burden of Royal Navy and Royal Marine combat casualties sustained in 11-years of operations in Iraq and Afghanistan. *J R Nav Med Serv* 2014;100(2):161-165
- Penn-Barwell JG, Bennett PM, Kay, A, Sargeant ID. Acute bilateral leg amputation following combat injury in UK servicemen. *Injury* 2014; 45(7):1105-1110.
- Penn-Barwell JG, Fries CA, Bennett PM, Midwinter MJ, Baker AB. The injury burden of recent combat operations: mortality, morbidity and return to service of UK Naval Service personnel following combat trauma. *Military Medicine* 2013; 178(11):1222-1226
- Bennett PM, Sargeant ID, Midwinter MJ, Penn-Barwell JG. Unilateral lower limb loss following combat injury: Medium-term outcomes in British military amputees. *Bone Joint J* 2013;95(2), 224
- Penn-Barwell JG, Bennett PM, Kendrew JM, Midwinter MJ, Richard RF. Severe open tibial fractures in combat trauma: management and preliminary outcomes. *Bone Joint J* 2013;95B:101-105
- Penn-Barwell JG, Fries CA, Sargeant ID, Bennett PM, Porter K. Aggressive soft tissue infections and amputation in military trauma patients. *J R Nav Med Serv* 2012;98(2):14-18
- Penn-Barwell JG, Bennett PM, Powers D, Standley D. Isolated hand injuries on operational deployment: an examination of epidemiology and treatment strategy. *Military Medicine* 2011; 176(12);1404-7
- Bennett P. A comparison of methods used for the prevention of venous thromboembolic disease among orthopaedic surgeons at Wolverhampton, United Kingdom and Auckland, New Zealand. *J R Nav Med Serv* 2009;95(2):81-88

APPENDICES

■ TRAUMA

Unilateral lower limb loss following combat injury

MEDIUM-TERM OUTCOMES IN BRITISH MILITARY AMPUTEES

P. M. Bennett,
I. D. Sargeant,
M. J. Midwinter,
J. G. Penn-Barwell

*From the Royal
Centre for Defence
Medicine,
Birmingham, United
Kingdom*

This is a case series of prospectively gathered data characterising the injuries, surgical treatment and outcomes of consecutive British service personnel who underwent a unilateral lower limb amputation following combat injury. Patients with primary, unilateral loss of the lower limb sustained between March 2004 and March 2010 were identified from the United Kingdom Military Trauma Registry. Patients were asked to complete a Short-Form (SF)-36 questionnaire. A total of 48 patients were identified: 21 had a trans-tibial amputation, nine had a knee disarticulation and 18 had an amputation at the trans-femoral level. The median New Injury Severity Score was 24 (mean 27.4 (9 to 75)) and the median number of procedures per residual limb was 4 (mean 5 (2 to 11)). Minimum two-year SF-36 scores were completed by 39 patients (81%) at a mean follow-up of 40 months (25 to 75). The physical component of the SF-36 varied significantly between different levels of amputation ($p = 0.01$). Mental component scores did not vary between amputation levels ($p = 0.114$). Pain ($p = 0.332$), use of prosthesis ($p = 0.503$), rate of re-admission ($p = 0.228$) and mobility ($p = 0.087$) did not vary between amputation levels.

These findings illustrate the significant impact of these injuries and the considerable surgical burden associated with their treatment. Quality of life is improved with a longer residual limb, and these results support surgical attempts to maximise residual limb length.

Cite this article: *Bone Joint J* 2013;95-B:224-9.

■ P. M. Bennett, MBChB, Royal Navy Medical Officer
■ I. D. Sargeant, OBE, FRCS(Tr & Orth), Consultant Orthopaedic Trauma Surgeon Royal Centre for Defence Medicine (RCDM), Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK.

■ M. J. Midwinter, CBE, MD, FRCS(Gen), Defence Professor Surgery, Consultant General Surgeon

■ J. G. Penn-Barwell, MBChB, MRCS, Trauma and Orthopaedic Registrar, Research Fellow Royal Centre for Defence Medicine (RCDM), National Institute for Health Research (NIHR), Surgical Reconstruction & Microbiology Research Centre (SRMRC), Birmingham, UK.

Correspondence should be sent to Surg Lt Cdr J. G. Penn-Barwell RN; e-mail: jowan@doctors.net.uk

©2013 The British Editorial Society of Bone & Joint Surgery
doi:10.1302/0301-620X.95B2.30365 \$2.00

Bone Joint J
2013;95-B:224-9.
Received 4 July 2012; Accepted after revision 31 October 2012

Modern high-energy military weapons produce devastating injuries predominantly affecting the extremities.^{1,2} Amputation of the lower limb, be it induced traumatically at time of injury or surgically if the limb is unsalvageable, is a life-changing injury.³

In civilian practice the main reason for limb amputation is vascular disease,⁴ but the military patient is different being younger, largely free from medical comorbidities and possessing a high level of pre-injury fitness.

This paper describes the injuries and surgical treatment of British service personnel who sustained a unilateral lower limb amputation following combat injury and defines their medium-term outcomes.

Patients and Methods

This study was registered with, and approved by, the Joint Medical Command. The United Kingdom military Joint Theatre Trauma Registry (JTTR) is an electronic database of prospectively gathered information on all casualties, collected by research nurses in deployed military medical facilities and at the Royal Centre for Defence Medicine (RCDM), Birmingham, United Kingdom.⁵ All patients

who required a trauma alert call either on presentation to a deployed military medical facility or subsequently require repatriation from such a facility to the United Kingdom were included in the study. The database is managed by the Academic Department for Military Emergency Medicine at RCDM and administered by the Defence Analytical Services and Advice.

The JTTR was searched for all patients with a unilateral lower limb amputation between March 2004 and March 2010. Patients were excluded if they underwent an amputation at the ankle or distally. Patients in whom amputation occurred more than seven days after injury were also excluded. Patients who consented to participate in the study were interviewed over the telephone and asked to complete the United Kingdom-adapted second version of the Short-Form 36 (SF-36 V2),^{6,7} with a reminder letter and second questionnaire sent at four weeks in the event of the initial form not being returned.

Responses to the SF-36 questionnaires were entered into the Quality Metric Health Outcomes Scoring Software version 4.0 (Quality Metric, Lincoln, Rhode Island). Patient

Table I. Analysis of characteristics of patients lost to follow-up, by Mann-Whitney U test for all characteristics except amputation level (Fisher's exact test)

	Included patients	Lost to follow-up	p-value
Patients (n)	39	9	
Mean (SD) age (yrs)	24.4 (5.16)	24.8 (3.4)	0.4992 [†]
Mean (SD) NISS	27.4 (13.9)	30.8 (15.9)	0.7336 [†]
Amputation level (n, %)*			0.5536 [‡]
TTA	18 (46)	3 (33)	
KD	8 (21)	1 (11)	
TFA	13 (33)	5 (56)	
Mean (SD) time since injury (mths)	39.6 (13.8)	50 (2.7)	0.2004 [†]

* TTA, trans-tibial; KD, knee disarticulation; TFA, trans-femoral

† Mann-Whitney U test

‡ Fisher's exact test

cohorts were defined according to the level of their amputation: trans-tibial (TT), knee-disarticulation (KD) or trans-femoral (TF). Descriptive data were presented as a mean or median with standard deviation (SD) and/or range.

The severity of the injury was measured using the New Injury Severity Score (NISS),⁸ which was calculated from the 2005 military edition of the Abbreviated Injury Scale coding manual.⁹ NISS is used in preference to the traditional Injury Severity Score (ISS),¹⁰ which only measures the most severe injuries from separate body regions. NISS methodology measures the most severe injuries, even if they occur in the same region, and more accurately quantifies trauma patterns from combat.^{8,11}

A perfused but probably unsalvageable limb may not be amputated in the immediate medical facility. This permits the final decision on amputation or salvage to be made by the orthopaedic and plastic surgeons at the RCDM and also retains a potential source of graft material. Planned amputation in these patients occurred at the third surgical episode, after the initial operation in the United Kingdom, which was used to assess tissue viability and to plan reconstruction or amputation. The delay until planned amputation was used for the basis of the seven day definition of primary amputation.

An intact or readily reconstructable knee joint, in which there was adequate bone length and soft-tissue cover, especially from skin and fascia below the knee, normally allowed for a successful TT amputation. It was often necessary to perform TT amputations at a level more proximal than the 8 cm of tibial length per 1 m of height, as has been described.¹²

For an amputation at the level of the knee joint, knee disarticulation was performed and the joint capsule and collateral structures secured over the femoral condyles in order to achieve a stable soft-tissue envelope, over which fat and skin could be closed. If sufficient joint capsule was not present as a result of the initial injury or due to previous debridement, the articular surface of the condyles was excised, either down to sub-chondral bone or bone levels similar to the resection undertaken in total knee replacement. This exposed cancellous bone encouraged faster tissue adherence than the articular cartilage when the capsule

was absent. Narrowing and shortening of the femur can allow primary closure with a 'pseudo-KD' rather than amputation at the TF level. These procedures were delayed until wound closure was possible, thus reducing the risk of femoral intramedullary bacterial colonisation. An intact patella was not routinely excised, although the articular surface was occasionally removed to reduce anterior-posterior bulk in order to facilitate wound closure, although if the patella had sustained a comminuted fracture it was excised rather than reconstructed.

TF amputations were performed at a level to maximise bone length without compromising the soft-tissue cover. If required in a mid-shaft amputation, several centimetres of bone would be sacrificed in order to cover the weight-bearing surface with healthy muscle and skin. Conversely, in the presence of a distal TF amputation with a good muscle envelope but extensive skin loss, split-skin grafting was used to achieve coverage, rather than converting to a higher TF amputation with cutaneous closure.

Gauze-based topical negative pressure (TNP) dressings were used at every stage of the surgical management of stumps, at all amputation levels and for several days after definitive closure. When further soft-tissue necrosis was anticipated after surgery, the tissues were left in their anatomical position in order to reduce the risk of damage and blood loss, while a level of demarcation formed.

Statistical analysis. Comparison across cohorts was performed using a Kruskal-Wallis test and between cohorts with a Mann-Whitney U test for continuous variables. Fisher's exact test was used for comparison of dichotomous data. A threshold for significance was set at a p-value < 0.05.

Results

A total of 1694 United Kingdom military personnel were injured or killed during this study period, of whom 48 patients (2.8%) had a primary unilateral lower limb amputation for which clinical notes and JTTR records were available. In all, 21 patients had a TT amputation, nine had a KD and 18 had amputation at the TF level. It was not possible to contact seven of the 48 patients. A further two patients provided information on the use of prostheses and pain but did not return their SF-36 questionnaires.

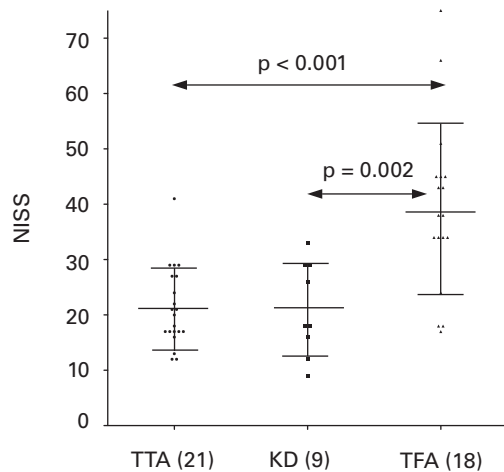


Fig. 1

Plot showing the mean New Injury Severity Score (NISS) by level of amputation (TTA, trans-tibial; KD, knee disarticulation; TFA, trans-femoral). The transverse lines represent the mean values and the error bars represent the standard deviation. There was a significant difference across all groups ($p < 0.001$, Kruskal-Wallis) and between the KD and TFA groups ($p = 0.002$, Mann-Whitney).

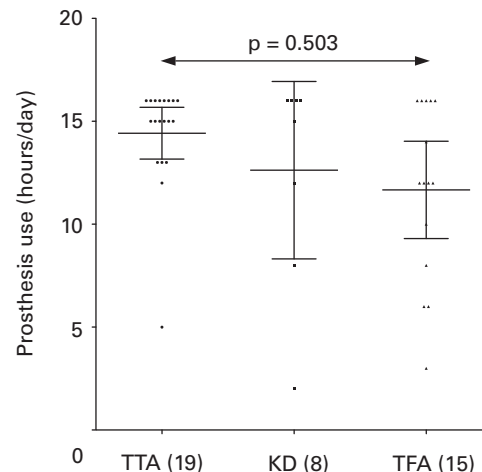


Fig. 3

Plot showing the mean duration of prosthesis use per day by level of amputation (TTA, trans-tibial; KD, knee disarticulation; TFA, trans-femoral). The transverse lines represent the mean values and the error bars represent the standard deviation. There was no significant difference across the groups ($p = 0.503$, Kruskal-Wallis).

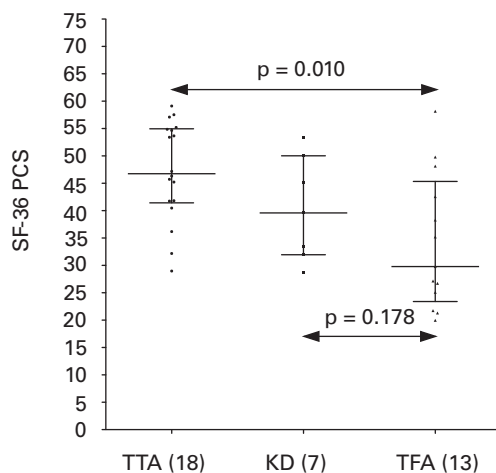


Fig. 2

Plot showing the mean Short-Form 36 physical component score (SF-36 PCS) by level of amputation (TTA, trans-tibial; KD, knee disarticulation; TFA, trans-femoral). The transverse lines represent the mean values and the error bars represent the standard deviation. There was a significant difference across all groups ($p = 0.010$, Kruskal-Wallis), and an observed difference between the KD and TFA groups ($p = 0.178$, Mann-Whitney), although this did not reach significance.

and the remainder were as a result of improvised explosive devices (IEDs). Only six patients were injured during service in Iraq, with the remainder injured during operations in Afghanistan.

Of the patients in this series, four had NISS scores > 15 , a commonly used definition of 'major-trauma'. The mean NISS in this study was 27 (9 to 75, SD 14), and the NISS was significantly higher in patients with more proximal amputation ($p < 0.001$, Kruskal-Wallis) (Fig. 1). The mean number of surgical procedures per limb was 4.1 (SD 1.8) and the mean critical care stay was 4.9 days (SD 6.3). Three patients had contralateral bony injuries of their hindfoot or ankle, seven had contralateral tibial fractures and one had a contralateral femoral fracture.

The physical component score (PCS) of the SF-36 declined significantly with more proximal amputation levels ($p = 0.01$, Kruskal-Wallis), but there was no significant difference between the TF and KD cohorts when compared directly ($p = 0.178$, Mann-Whitney) (Fig. 2). Mental component scores did not vary across groups ($p = 0.114$, Kruskal-Wallis). The mean self-reported prosthesis use, in hours per day, was not significantly different across the groups ($p = 0.503$, Kruskal-Wallis). In the TT group, prosthetic use was a mean 14.4 hours per day (SD 2.5) (Fig. 3).

The self-declared ability to walk 500 m did not vary significantly across the amputation groups ($p = 0.087$, Fisher's exact test) (Fig. 4).

There was no significant difference between amputation levels in the 20 of the 42 patients reporting residual limb pain ($p = 0.8$, Fisher's exact test). There was similarly no significant difference between amputation level with

Therefore full data were available on 39 of the 48 patients (81%) (Table I). The mean follow-up period was 40 months, (25 to 75; SD 16).

All but one of the patients was male and all of the injuries were the result of blast weapons; nine blasts were as a result of indirect fire munitions (e.g., rocket-propelled grenades)

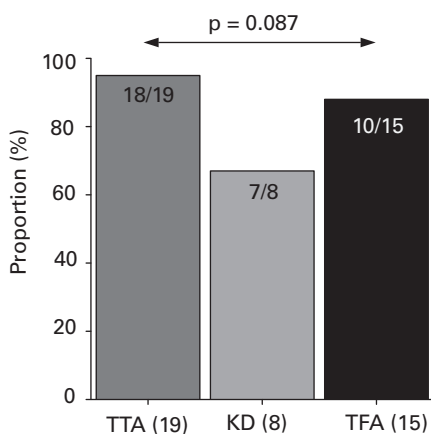


Fig. 4

Bar chart showing the proportion of patients with a self-declared ability to walk more than 500 m by amputation level (TTA, trans-tibial; KD, knee disarticulation; TFA, trans-femoral). There was no statistically significant difference between the groups ($p = 0.087$, Fisher's exact test).

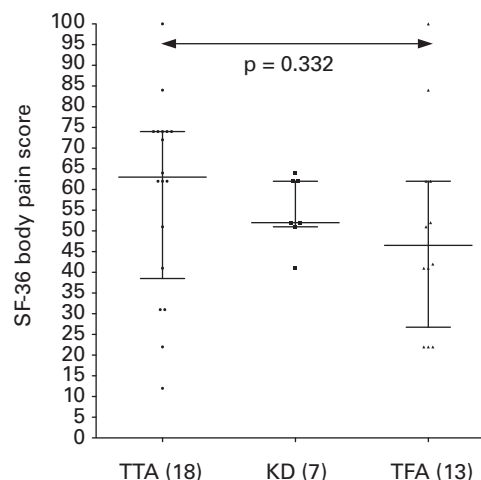


Fig. 5

Plot showing the mean Short-Form (SF)-36 bodily pain score by level of amputation (TTA, trans-tibial; KD, knee disarticulation; TFA, trans-femoral). The transverse lines represent the mean values and the error bars represent the standard deviation. No significant difference was found between the groups ($p = 0.332$, Kruskal-Wallis).

respect to the bodily pain component of the SF-36 ($p = 0.332$, Kruskal-Wallis) (Fig. 5).

Unplanned re-admission for a complication related to the residual limb occurred in 19 of 41 patients (46%). There was no association between re-admission and the level of amputation ($p = 0.228$, Fisher's exact test).

Discussion

The results show that lower-limb loss represents a significant, life-changing burden on survivors of combat injury and that longer residual limb lengths are associated with higher physical component of quality of life scores.

Currently for amputees there is no single accepted outcome measurement.^{13,14} The SF-36 was developed as a non-specific measure of the impact of illness or disability on the quality of life.⁶ It was used in this study due to its extensive application in outcome studies looking at both amputation and limb salvage following trauma, and also because it could differentiate between the physical and emotional effect of injuries on the patient's quality of life.

The ability to walk 500 m has been found to be a key factor for independent living,¹⁵ and was therefore assessed in our study. Employment was not evaluated as it is not considered relevant in the military context, because many service personnel in this study were still employed in the services, either completing their rehabilitation or retained in a non-combat role.

The surgical strategy for limb amputation has evolved during the recent conflicts in Iraq and Afghanistan. Military surgeons in previous wars performed limb amputation proximal to the zone of injury in order to allow for the formation of a robust stump with closure of healthy wound

edges,¹⁶ typically requiring only two surgical procedures.¹⁷ In the current conflicts, the surgical strategy has focused on maximising the length of the residual limb. This has been achieved by serial debridement over several operations where only non-viable tissue from within the zone of injury is excised. Plastic surgical techniques are then used to ensure closure of the soft-tissue envelope over bone. This approach results in a longer residual limb, albeit with a less regular surface. The authors believe this approach gives a better long-term functional result for amputees but it does require a greater number of surgical procedures and longer hospital stay.

SF-36 outcomes have previously been described in military patients with unilateral lower limb loss but not distinguishing between amputation levels.¹⁷⁻²⁰ Studies with medium- and long-term follow-up were identified during a systematic review²¹ and the authors contacted for unpublished data enabling outcomes by amputation height to be calculated (Table II).

However direct comparison between the results of this study and previous data is difficult. Gunawardena et al¹⁸ and Taghipour et al¹⁹ excluded all patients with other significant injuries. Dougherty's¹⁷ patient population of Vietnam veterans is the most comparable to the patients in this study, but the 28-year follow-up is likely to affect outcomes, as even two years after injury, a few patients in our study were still having surgical treatment to their residual limb.

The question of amputation through the knee remains unresolved. The British Association of Plastic, Reconstructive and Aesthetic Surgeons/British Orthopaedic Association (BAPRAS/BOA) standards for managing open lower

Table II. Case series of unilateral lower limb amputees

Author/s	Conflict	Nationality of Patients	Follow-up (yrs)	Amputation level*	n	Mean (sd) SF-36 PCS†
Dougherty ^{17,24}	US-Vietnam War	US	28	TT	123	61
				TF	46	51
Gunawardena et al ¹⁸	Sri-Lankan Civil War	Sri-Lankan	4	TT	416	62 (9.6)
				TF	48	53 (11.5)
Taghipour et al ¹⁹	Iran-Iraq War	Iranian	22	TT	60	46 (13.3)
				KD	38	46 (13.4)
				TF	43	46 (18.2)

* TT, trans-femoral; KD, knee disarticulation; TF, trans-femoral

† SF-36 PCS, Short-Form 36 Physical Component Score

limb fractures did not recommend KD in adults.²⁰ This was based on the 16 patients with KD in the Lower-Extremity Assessment Project in which they found significantly better sickness impact profile scores and walking speeds in patients with TF amputations compared with KD at two years.³ An alternative view is presented in the findings of a recent meta-analysis of outcomes after amputation following trauma, which showed that patients with a KD performed better than TF amputation with regard to SF-36 PCS and walking distance.²¹

Our findings support the position that the quality of life is largely related to the performance of the functional unit formed by the combination of the prosthesis and the residual limb. However, prosthetic fitting for KD amputations is technically more challenging than at the TF level, and surgeons operating in countries with a limited limb-fitting service should take this into account when deciding on the level of an amputation.¹⁶

Combat injuries are inherently heterogeneous and grouping them by amputation height ignores these differences, particularly injuries to the contralateral limb. The correlation between amputation height and SF-36 PCS shows that the mechanical advantage of a longer residual limb and the higher energy demand associated with walking on a shorter residual limb is confirmed.²² However, patients with more proximal amputations may also have more severe injuries; our study is not large enough to allow for analysis of the relationship between amputation height and SF-36 PCS, while taking into account the severity of the injury. TF amputation in this population can refer to a wide range of amputation heights, including extreme proximal levels. Delayed amputation has been excluded from this study, as the authors regard this as a failure of limb salvage and believe that this is a clinical entity distinct from primary amputation. This is because amputation often occurs months or even years after injury, during which time the patient's rehabilitation has stalled and multiple operations may be required with associated complications.²³

Despite these weaknesses, this study describes the injuries and treatments of all British Service personnel with a

primary unilateral lower limb amputation sustained over a six-year period of combat operations. Furthermore, we present the outcomes in 39 patients with a mean follow-up of 40 months. These findings show that superior quality of life outcomes occur in patients with longer residual limbs, which supports the current surgical strategy of maximising residual limb length whenever possible.

On behalf of the Severe Lower Extremity Combat Trauma (SeLECT) Study Group: Surg Lt Cdr J. G. Penn-Barwell, Surg Lt P. M. Bennett, Surg Lt Cdr C. A. Fries, Wg Cdr J. M. Kendrew, Surg Capt M. Midwinter, Surg Cdr R. F. Rickard, Gp Capt I. D. Sargeant, Professor K. Porter, Lt Col T. Rowlands, Lt Col A. Mountain, Lt Col T. Cubison, Mr J. Cooper, Mr D. Wallace, Mr D. Power, Lt Col S. Jeffery, Wg Cdr D. Evriadiades, Wg Cdr W. J. C. van Niekerk, Col A. Kay L/RAMC, SRMRC/RCDM/Queen Elizabeth Hospital, Birmingham, United Kingdom.

The authors would like to thank Mrs J. Beach for helping to collect and analyse the study results and Mr S. Banwell for assisting in data collection. The Academic Department of Military Emergency Medicine (ADMED), Defence Analytical Services and Advice (DASA) are thanked for collecting, collating and identifying the appropriate data for this paper. The authors would also like to thank Professor N. Gunawardena MD, University of Colombo, Sri Lanka, Dr H. Taghipour MD and Dr Y. Moharamzad MD, Baqiyatallah Medical Sciences Centre, Iran, for generously sharing unpublished data from their previous work in this field.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Ministry of Defence or Her Majesty's Government.

All of the authors are serving officers in the Royal Navy and Royal Air Force and the copyright of this work remains with the crown.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

- Owens BD, Kragh JF Jr, Macaitis J, Svoboda SJ, Wenke JC. Characterization of extremity wounds in Operation Iraqi Freedom and Operation Enduring Freedom. *J Orthop Trauma* 2007;21:254–257.
- Ramasamy A, Harrison SE, Stewart MP, Midwinter M. Penetrating missile injuries during the Iraqi insurgency. *Ann R Coll Surg Engl* 2009;91:551–558.
- MacKenzie EJ, Bosse MJ, Castillo RC, et al. Functional outcomes following trauma-related lower-extremity amputation. *J Bone Joint Surg [Am]* 2004;86-A:1636–1645.
- Dillingham TR, Pezzin LE, MacKenzie EJ. Limb amputation and limb deficiency: epidemiology and recent trends in the United States. *South Med J* 2002;95:875–883.
- Smith J, Hodgetts T, Mahoney P, et al. Trauma governance in the UK defence medical services. *J R Army Med Corps* 2007;153:239–242.
- Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I: conceptual framework and item selection. *Med Care* 1992;30:473–483.
- Jenkinson C, Stewart-Brown S, Petersen S, Paice C. Assessment of the SF-36 version 2 in the United Kingdom. *J Epidemiol Community Health* 1999;53:46–50.
- Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *J Trauma* 1997;43:922–925.
- No authors listed. *The Abbreviated Injury Scale 2005: Military*. Des Plaines: Association for the Advancement of Automotive Medicine, 2005.

10. **Baker SP, O'Neill B, Haddon W Jr, Long WB.** The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 1974;14:187–196.
11. **Champion HR, Holcomb JB, Lawnick MM, et al.** Improved characterization of combat injury. *J Trauma* 2010;68:1139–1150.
12. **Bulstrode CJK.** *Oxford textbook of orthopedics and trauma*. New York: Oxford University Press, 2002:97.
13. **Rommers GM, Vos LD, Groothoff JW, Eisma WH.** Mobility of people with lower limb amputations: scales and questionnaires: a review. *Clin Rehabil* 2001;15:92–102.
14. **Hays RD, Hahn H, Marshall G.** Use of the SF-36 and other health-related quality of life measures to assess persons with disabilities. *Arch Phys Med Rehabil* 2002;83(Suppl):S4–S9.
15. **Geertzen JH, Bosmans JC, van der Schans CP, Dijkstra PU.** Claimed walking distance of lower limb amputees. *Disabil Rehabil* 2005;27:101–104.
16. **Coupland RM.** Amputation for antipersonnel mine injuries of the leg: preservation of the tibial stump using a medial gastrocnemius myoplasty. *Ann R Coll Surg Engl* 1989;71:405–408.
17. **Dougherty PJ.** Transtibial amputees from the Vietnam War: twenty-eight-year follow-up. *J Bone Joint Surg [Am]* 2001;83-A:383–389.
18. **Gunawardena NS, Seneviratne Rde A, Athauda T.** Functional outcomes of unilateral lower limb amputee soldiers in two districts of Sri Lanka. *Mil Med* 2006;171:283–287.
19. **Taghipour H, Moharamzad Y, Mafi AR, et al.** Quality of life among veterans with war-related unilateral lower extremity amputation: a long-term survey in a prosthesis center in Iran. *J Orthop Trauma* 2009;23:525–530.
20. **Nanchahal J, Nayagam S, Khan U, et al.** *Standards of the management of open fractures of the lower limb*. London: Royal Society of Medicine Press, 2009.
21. **Penn-Barwell JG.** Outcomes in lower limb amputation following trauma: a systematic review and meta-analysis. *Injury* 2011;42:1474–1479.
22. **Waters RL, Perry J, Antonelli D, Hislop H.** Energy cost of walking of amputees: the influence of level of amputation. *J Bone Joint Surg [Am]* 1976;58-A:42–46.
23. **Lange RH, Bach AW, Hansen ST Jr, Johansen KH.** Open tibial fractures with associated vascular injuries: prognosis for limb salvage. *J Trauma* 1985;25:203–208.
24. **Dougherty PJ.** Long-term follow-up of unilateral transfemoral amputees from the Vietnam war. *J Trauma* 2003;54:718–723.

■ TRAUMA

The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period

P. M. Bennett,
I. D. Sargeant,
R. W. Myatt,
J. G. Penn-Barwell

From Royal Centre
for Defence
Medicine, Queen
Elizabeth Hospital,
Edgbaston,
Birmingham, United
Kingdom

This is a retrospective study of survivors of recent conflicts with an open fracture of the femur. We analysed the records of 48 patients (48 fractures) and assessed the outcome. The median follow up for 47 patients (98%) was 37 months (interquartile range 19 to 53); 31 (66%) achieved union; 16 (34%) had a revision procedure, two of which were transfemoral amputation (4%).

The New Injury Severity Score, the method of fixation, infection and the requirement for soft-tissue cover were not associated with a poor outcome. The degree of bone loss was strongly associated with a poor outcome ($p = 0.00204$). A total of four patients developed an infection; two with *S. aureus*, one with *E. coli* and one with *A. baumannii*.

This study shows that, compared with historical experience, outcomes after open fractures of the femur sustained on the battlefield are good, with no mortality and low rates of infection and late amputation. The degree of bone loss is closely associated with a poor outcome.

Cite this article: *Bone Joint J* 2015;97-B:842–6.

In civilian practice, open fractures of the femur are relatively uncommon. Compared with fractures at other sites they are associated with higher energy and are more severe injuries.¹ On the battlefield, open femoral fractures were often fatal in the past. In the First World War, the rate of mortality from a fracture of the femur caused by a gunshot was as high as 80%.²

In modern warfare, the extremities are more commonly involved in combat injuries than other regions of the body.³ Recent research has shown a significant improvement in survival over the course of the conflicts in Iraq and Afghanistan.⁴ This has resulted in the survival of casualties who would have succumbed to their limb injuries in previous conflicts. The long-term outcome of open femoral fractures is, therefore, increasingly important.

The aim of this study was to characterise severe open femoral fractures in survivors of recent conflicts and to identify factors associated with poor healing.

Patients and Methods

This study was registered with, and approved by, the Joint Medical Command. The Joint Theatre Trauma Registry (JTTR) is an electronic database that prospectively gathers data on casualties sustained overseas and is administered by Defence Statistics.⁵ Data are gathered

on all casualties, including soldiers who are killed immediately, soldiers whose injuries trigger a trauma alert on arrival at a deployed medical facility, and soldiers whose injuries subsequently require repatriation to the United Kingdom. The data are collected by research nurses at the medical facilities and at the Royal Centre for Defence Medicine (RCDM), Birmingham, United Kingdom.⁵

The registry was searched for codes that described bony injuries of the hip, femur or knee sustained between the invasion of Iraq on 19 March 2003 and 31 December 2012. Only survivors from the United Kingdom with AO type 32 open fractures were included.⁶ Clinical notes and radiographs were reviewed; we included patients with a lower limb amputation if the limb was amputated at or below the knee. We excluded patients with a transfemoral amputation and more proximal femoral fracture, and those with follow-up of less than 12 months. The clinical notes and radiographs were reviewed, and the Ministry of Defence primary care electronic records (Defence Medical Information Capability Programme (DMICP)). Data were gathered on the patients' demographics, the details of the injury and management.

A good outcome was considered to be a fracture that clinically and radiologically achieved union without requiring further

■ P. M. Bennett, MB ChB, MRCS, Royal Navy Medical Officer
■ I. D. Sargeant, OBE, FRCS (Tr&Orth), Royal Air Force Consultant Orthopaedic Trauma Surgeon
■ J. G. Penn-Barwell, MB ChB, MSc, MRCS, Royal Navy Trauma & Orthopaedic Registrar
Royal Centre for Defence Medicine, ICT Research Park, Vincent Drive, Edgbaston, Birmingham B15 2SQ, UK.

■ R. W. Myatt, BM BS, Royal Navy Medical Officer
42 Commando Royal Marines, Bickleigh Barracks, Plymouth, Devon, UK.

Correspondence should be sent to Surg Lt Cdr J. G. Penn-Barwell RN;
e-mail: jowanpb@me.com

©2015 The British Editorial Society of Bone & Joint Surgery
doi:10.1302/0301-620X.97B6.34962 \$2.00

Bone Joint J
2015;97-B:842–6.

Received 14 August 2014;
Accepted after revision 16 January 2015

Table I. Grading system for bone loss

Grade	Definition
0	None
1	Minimal, some bone loss but < 1 cm longitudinally around > 50% of the circumference of the shaft some cortical contact maintained
2	Moderate, bone loss between 1 cm and 2 cm around > 50% of the circumference of the shaft but with some cortical contact
3	Severe, bone loss > 2 cm around > 50% of the circumference of the shaft but with some cortical contact
4	Segmental bone loss

Table II. Mechanism of injury

Mechanism	n (%)
Gunshot wounds	25 (52)
Explosive – improvised explosive device	19 (40)
Explosive – indirect fire weapon e.g. mortar	3 (6)
Other	1 (2)

Table IV. Type of revision procedure with median time to revision and range shown

Procedure	n	Median time to revision (mths) (IQR)
Revision of fixation	11	4 (1 to 22)
Osteotomy and revision of fixation	3	12 (4 to 19)
TFA	2	12 (1 to 23)

IQR, interquartile range; TFA, trans-femoral amputation

Table III. Causes of revision with median time to revision and range

Cause	n	Median time to revision (mths) (IQR)
Metal failure	4	2 (1 to 4)
Malunion	4	16 (4 to 22)
Non-union	2	4 (3 to 12)
Infection	4	10 (4 to 15)
Other	2	12 (1 to 23)

IQR, interquartile range

surgery. Amputation or revision of the bony construct for non- or malunion were considered poor outcomes. Removal of symptomatic metalwork was not regarded as a negative outcome.

The following variables were considered: the New Injury Severity Score (NISS);⁷ the method of fixation, dichotomised into intramedullary nail or plates and screws; deep infection, defined as surgical treatment for an infected limb; the degree of bone loss, graded according to a previously described classification system (Table I)⁸ and the soft-tissue cover (where more than one plastic surgical technique was used the highest on the 'reconstructive ladder' was recorded).⁹

Statistical analysis. Descriptive data are presented as a median with interquartile range (IQR). Comparison between cohorts was performed with a Mann–Whitney U test for continuous variables or a Fisher's exact test for dichotomous variables, and with respect to bone loss, a 2 × 5 contingency table was used. When determining statistical significance, a Bonferroni correction was applied to reduce the chance of a type 1 error inherent in the comparison of multiple variables; significance was therefore set at $p < 0.01$. Analysis was performed using Graph Pad Prism (San Diego, California).

Results

There were 2184 wounded casualties during the study period. A search of the JTTR database identified 149 patients with bony injuries affecting the hip, femur or knee. A total of 101 did not meet the inclusion criteria, leaving 48

patients (48 open femoral fractures) in the study, representing 2.2% of all casualties during this period. No patient died following an isolated open femur fracture.

The median age of the patients was 23 years (IQR 20 to 29) and all but one were male. Most were injured as a result of gunshot wounds ($n = 25$, 52%, Table II).

Five patients had an amputation distal to the femoral fracture; three through the knee and two at a transtibial level. The fractures extended into the knee joint in seven patients. Six required arterial repair (Gustillo-Anderson IIC¹⁰) and eight required surgical treatment of an associated nerve injury. Five suffered a traumatic amputation of the contralateral leg.

Complete follow-up data were available for 47 patients (98%); further outcome analysis was performed on this cohort. The median follow-up was 37 months (IQR 19 to 53). Following initial surgical management, union of the femoral fracture was achieved without further intervention in 31 of these 47 patients (66%). Union was achieved in all non-amputee patients at a median of five months (IQR 4 to 7) post-operatively.

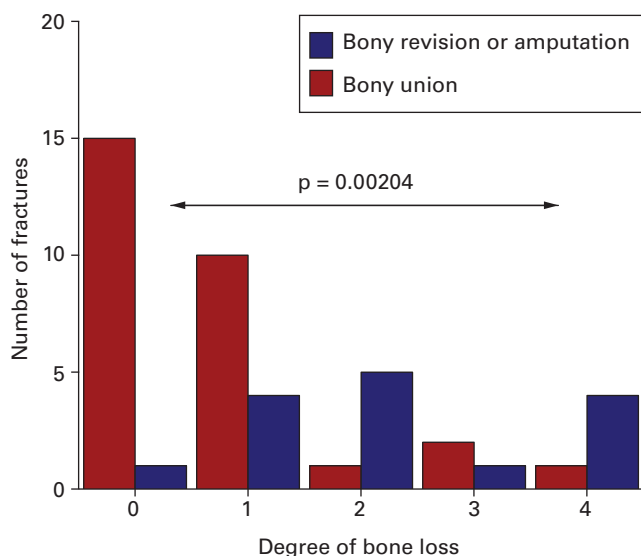
A total of 16 (34%) underwent further surgery; two required transfemoral amputation (4%) (Tables III and IV). There were no disarticulations of the hip. The median time from injury to the first revision procedure was four months (IQR 3.5 to 13.5).

The most common initial method of stabilisation was skeletal traction, used in 23 patients (48%). External fixation was used in 14 (29%) and the remaining fractures were splinted with a plaster cast or a Thomas splint with skin traction.

Definitive fixation was achieved with an intramedullary nail in 29 patients (60%) and 18 (38%) were managed with plates and/or screws. The median delay to fixation was three days (IQR 2.5 to 4.0), although one patient remained so physiologically unstable that he did not undergo fixation for 50 days. The median number of surgical episodes per femur was 4 (IQR 3.0 to 5.0). There was no association between the method of fixation and likelihood of union ($p = 0.5156$, Fisher's exact test).

Table V. Plastic surgical techniques for wound coverage and closure

Technique	n (%)
Delayed primary closure	20 (42)
Split skin graft	24 (50)
Local flap	2 (4)
Free flap	1 (2)

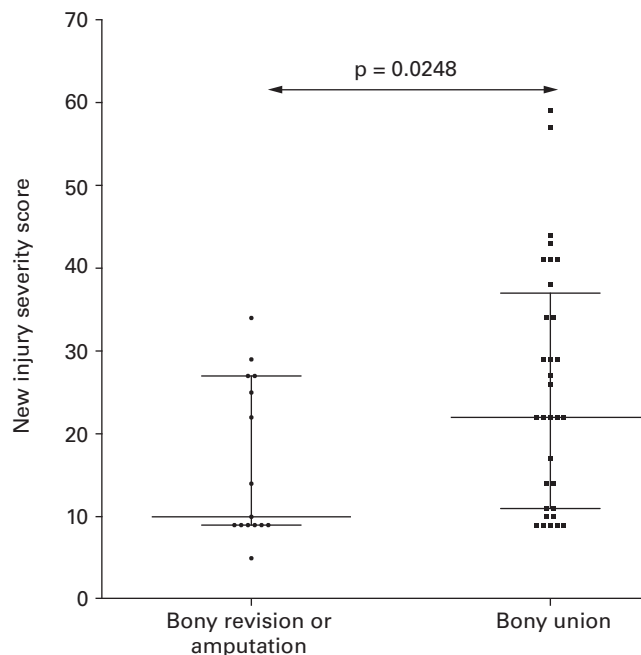
**Fig. 1**

Bar graph showing bone loss and outcome. P-value according to Fisher's exact test across all groups is shown.

Deep infection requiring surgical treatment occurred in four fractures (8%). In two patients, *Staphylococcus aureus* was isolated; in one, *Escherichia coli* and *Acinetobacter baumannii* was isolated in one patient. All four required further surgery before achieving union. Meaningful statistical analysis of such small numbers is limited, however there was no association between the presence of deep infection and outcome ($p = 0.0102$, Fisher's exact test).

Of the 47 patients with complete follow-up data, we were unable to categorise bone loss in three cases, as there were no initial radiographs available for review. Two of these cases had a good outcome and one had a poor outcome. Of the 44 with full follow-up and available initial radiographs, 61% (27/44) had associated bone loss. The degree of femoral bone loss was significantly associated with the requirement for further surgery ($p = 0.00204$, Fisher's exact test) as shown in Figure 1

The most common techniques used to cover or close wounds were split skin grafts and delayed primary closure Table V. Local or free flaps were required in three patients; this was not associated with outcome ($p = 0.2076$, Fisher's exact test).

**Fig. 2**

Scatter plot showing the New Injury Severity Score according to outcome. The horizontal lines denote the median and inter-quartile ranges. P-value, according to Mann-Whitney analysis, is shown.

The median NISS was 22 (IQR 9 to 34); 28 patients (58.3%) had a NISS > 15, the commonly used definition of major trauma.¹¹ There was no significant difference in NISS scores between patients with a good or poor outcome ($p = 0.0248$, Mann-Whitney analysis) (Fig. 2).

Discussion

This study characterises open femoral fractures sustained by survivors of recent conflict. There were no deaths due to an isolated femoral fracture during the study period, although it is likely that some of the multiply-injured casualties who were killed in action, or soon after injury, had an open fracture of the femur. However, as the aim of this paper was to examine the effect of variables on outcome, we only considered survivors of an open femoral fracture.

In the initial stages of the First World War, 100 years ago, an open femoral fracture was regarded as a fatal injury.^{12,13} Stabilisation of the fracture was so difficult in this era that some military surgeons suggested transfemoral amputation as the only method to reduce mortality rates.¹⁴ Following the introduction of the Thomas splint, Sir Henry Gray observed a reduction in mortality from 80% to 16%.¹⁵ Although the accuracy of this claim has been questioned as this reduction is likely to have been the result of improvements in many elements of care, it is clear that over the course of the First World War the management, and therefore outcome, of open femoral fractures, improved substantially.

The measure of improvement in the management of open femoral fractures at that time was mortality. As mortality

rates have fallen, infection has become a more important indicator of the success of management. In the Second World War, Soto-Hall and Horwitz¹⁶ reported deep infection in 27 of 100 open femoral fractures. In the Balkan conflict of the 1990s Miric et al¹⁷ described a deep, chronic infection rate of 12% in a series of 17 patients, while in a contemporary United States military cohort of 41 open proximal femur fractures, Mack et al¹⁸ reported a combined superficial and deep rate of infection of 31%.

Defining infection is not without difficulty. All combat wounds are on a spectrum of contamination-colonisation-infection. This study therefore used the unambiguous definition of infection as that requiring surgical treatment, as in similar studies.^{8,19} It is worth reiterating that the mainstay of treatment for wound sepsis remains surgical, as it was prior to the development of antibiotics, with excision of necrotic or heavily contaminated tissue and irrigation with saline. Other studies reporting infection rates in open fractures have either defined infection microbiologically,²⁰ clinically²¹ or have not stated how it was defined.²²

Only four patients (8%) in this series had a deep infection. We believe that this low rate of infection supports the current anti-microbial treatment strategy. Current military practice in the United Kingdom is to treat open fractures with 1.2 g of intravenous co-amoxiclav every eight hours, started as soon as possible after the point of wounding. If patients show signs of wound sepsis antibiotic therapy is tailored to the microbiological results. In those without definitive microbiological results, individual patients are discussed at a multi-disciplinary team meeting. In these situations, clindamycin is often given in addition to co-amoxiclav. However, in patients in whom the injury was sustained in an aquatic environment (e.g. irrigation canals), ciprofloxacin may also be administered to cover for atypical gram negative species (e.g. *Aeromonas hydrophila*). Antibiotics are discontinued when, in the opinion of the treating surgeon, the wound is free from signs of infection and regarded as healthy. This is typically longer than the three days or time of closure as suggested by guidelines from the British Association of Plastic Reconstructive and Aesthetic Surgeons/British Orthopaedic Association.²³

Open femoral fractures are less common than open tibial fractures and there are relatively few case series in the literature. This rate of infection is substantially lower than in a comparable cohort of open tibial fractures where the rate was 23%.⁸ Others have also reported that open tibial fractures are more prone to infection than femoral fractures,^{10,24} probably because the precarious blood supply of the subcutaneous tibia which is at risk of devitalisation at the time of injury or subsequent debridement.²⁴ Furthermore, it is probable that if the injury is sustained stepping onto a victim-initiated explosive device the lower leg will be more heavily contaminated with debris than the thigh, given the mechanics of a blast wave and the tibia's proximity to the epicentre.

The presence of deep infection was the sole predictor of poor outcome in a previously published, similar cohort of tibial fractures.⁸ We did not find a statistically significant association between deep infection and outcome in our cohort of open femoral fractures, although the small numbers involved limit the validity of the analysis. None of those with an infection healed without further surgery.

We found that the degree of loss of femoral bone significantly affected the outcome. The mechanism of injury for open femoral fractures is almost equally distributed between gunshot wounds (56%) and blasts (44%). This is in contrast to an equivalent cohort of open tibial fractures where there was a heavy preponderance of blast injuries (71%) to gunshot wounds (29%).⁸

In the tibia, a blast that causes much tibial bone loss without traumatic amputation would have devastating periosteal stripping and soft-tissue loss; the consequent devitalisation often precludes any attempt at limb salvage. Conversely, the soft-tissue envelope of the femur allows salvage to be a more viable option, even in open fractures with grade 3 or 4 bone loss. It is now our strategy to acutely shorten the femur in patients with grade 3 or 4 bone loss, followed by lengthening once bony union has been achieved, which was performed successfully in the most recent two cases of severe bone loss. We found a significantly higher risk of nonunion with grade 3 or 4 bone loss than with grade 2 bone loss. This is most likely to be due to the loss of the protective soft-tissue envelope and devitalisation of the periosteum sustained in the initial trauma.

The degree of contamination associated with blast wounds cannot be over-emphasised. The blast wave tends to drive debris deep into tissue planes, often extending far beyond that of visible damage. The two patients with open femoral fractures who subsequently required transfemoral amputation were injured by blasts, although this is not statistically significant. Similarly, in the cohort of tibial fractures, all seven patients who required transtibial amputation had been injured in blasts.⁸ Again, this did not reach significance.

In civilian practice, 40% of open fractures occur in the lower limb, although most of these are tibial fractures with an approximate ratio for open fractures of 4:1 tibia to femur.¹ In our study period of ten years, open femoral fractures were seen in 2.2% of all casualties. Over the same period in Iraq open tibial fractures occurred in 4.6% of all casualties, confirming the observation that open tibial injuries are more common than those affecting the femur in whichever setting.³

In general, decisions regarding stabilisation of the fracture in the military patient are the same as for the civilian patient. However, in circumstances where a severely traumatised patient has an extensive wound facilitating access to the fracture, plate fixation is used. This explains the relatively common use of plates in our series, relative to IM nails. Typically unreamed, solid IM nails are used to reduce the surgical insult and operating time.

The study has limitations, being retrospective and observational in nature. The relatively small cohort of patients with multiple injuries prevents direct comparison between groups. Ideally patient-reported outcome measures would be collected, but as Figure 1 shows, these patients typically have many injuries and this would be a significant confounder for overall quality-of-life outcome tools. We believe that using both primary and secondary care records to gather follow-up data strengthens the outcome data obtained.

The Lower Extremity Assessment Project showed that re-admission to hospital closely correlates with a poor outcome.²⁵ We therefore chose revision surgery, including delayed amputation, as an indicator of failure of treatment.

Despite the weaknesses, we believe that this study successfully details the injuries, treatment and initial outcomes in military casualties with an open fracture of the femur sustained over a decade of conflict.

Author Contributions:

P. M. Bennett: Initial data collection, Manuscript preparation.

I. D. Sargeant: Study design, Clinical oversight.

R. W. Myatt: Follow-up data collection.

J. G. Penn-Barwell: Study design, Data collection, Data analysis, Manuscript preparation.

On behalf of the Severe Lower Extremity Combat Trauma (SeLECT) Study Group: Surg Lt Cdr JG Penn-Barwell, Surg Lt PM Bennett, Surg Lt Cdr CA Fries, Wg Cdr JM Kendrew, Surg Capt M Midwinter, Dr J Bishop, Surg Capt RF Rickard, Gp Capt ID Sargeant OBE, Prof Sir K Porter KBE, Lt Col T Rowlands, Lt Col A Mountain, Lt Col S Jeffrey, Dr D Mortiboy, Mr AFG Groom, Surg Lt RW Myatt, Surg Capt SA Stapley, Lt Col M Foster, Col A Kay.

© Crown Copyright 2015.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Ministry of Defence or Her Majesty's Government.

All of the authors are serving officers in the Royal Navy or Royal Air Force and the copyright of this work remains with the crown. Clinical Information and Exploitation Team, Joint Medical Command and UK Defence Statistics (Health) are thanked for collecting, collating and identifying the appropriate data for this paper.

The professionalism of the Medical, Nursing and support staff of the Defence Medical Services and University Hospitals Birmingham for their work in treating the patients described in this study is gratefully acknowledged.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

This article was primary edited by E. Moulder and first proof edited by J. Scott.

References

1. Court-Brown CM, Rimmer S, Prakash U, McQueen MM. The epidemiology of open long bone fractures. *Injury* 1998;29:529–534.

2. Brav EA. Military contributions to the development of orthopaedic surgery by the Armed Forces, U.S.A. Since World War I. *Clin Orthop Relat Res* 1966;44:115–126.
3. Owens BD, Kragh JF Jr, Wenke JC, et al. Combat wounds in operation Iraqi Freedom and operation Enduring Freedom. *J Trauma* 2008;64:295–299.
4. Penn-Barwell JG, Roberts SA, Midwinter MJ, Bishop JR. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003–2012. *J Trauma Acute Care Surg* 2015;78:1014–1020.
5. Smith J, Hodgetts T, Mahoney P, et al. Trauma governance in the UK defence medical services. *J R Army Med Corps* 2007;153:239–242.
6. Müller ME, Nazarian S, Koch P. Classification AO des fractures. Tome I. Les os longs. 1987, Berlin: Springer-Verlag.
7. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *J Trauma* 1997;43:922–925.
8. Penn-Barwell JG, Bennett PM, Fries CA, et al. Severe open tibial fractures in combat trauma: Management and preliminary outcomes. *Bone Joint J* 2013;95-B:101–105.
9. Boyce DE, Shokrollahi K. Reconstructive surgery. *BMJ* 2006;332:710–712.
10. Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. *J Bone Joint Surg [Am]* 1976;58-A:453–458.
11. Boyd CR, Tolson MA, Copes WS. Evaluating trauma care: the TRISS method. *J Trauma* 1987;27:370–378.
12. MacBean Ross JN. With a Royal Marine Battalion in France. *J R Nav Med Serv* 1917;3:465–471.
13. Penn-Barwell JG. Commentary on "with a Royal Marine battalion in France". 1917. *J R Nav Med Serv* 2014;100:117–122.
14. Robinson PM, O'Meara MJ. The Thomas splint: its origins and use in trauma. *J Bone Joint Surg [Br]* 2009;91-B:540–544.
15. Gray HMW. *The Early Treatment of War Wounds*. London: Henry Frowde and Hodder and Stoughton, 1919.
16. Soto-Hall R, Horwitz T. The treatment of compound fractures of the femur. *J Am Med Assoc* 1946;130:128–134.
17. Miric DM, Bumbasirevic MZ, Senohradski KK, Djordjevic ZP. Pelvifemoral external fixation for the treatment of open fractures of the proximal femur caused by firearms. *Acta Orthop Belg* 2002;68:37–41.
18. Mack AW, Freedman BA, Groth AT, et al. Treatment of open proximal femoral fractures sustained in combat. *J Bone Joint Surg [Am]* 2013;95-A:13.
19. Burns TC, Stinner DJ, Mack AW, et al. Microbiology and injury characteristics in severe open tibia fractures from combat. *J Trauma Acute Care Surg* 2012;72:1062–1067.
20. Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. *Clin Infect Dis* 2007;45:409–415.
21. Dellinger EP, Caplan ES, Weaver LD, et al. Duration of preventive antibiotic administration for open extremity fractures. *Arch Surg* 1988;123:333–339.
22. Ashford RU, Mehta JA, Cripps R. Delayed presentation is no barrier to satisfactory outcome in the management of open tibial fractures. *Injury* 2004;35:411–416.
23. Naique SB, Pearse M, Nanchahal J. Management of severe open tibial fractures: the need for combined orthopaedic and plastic surgical treatment in specialist centres. *J Bone Joint Surg [Br]* 2006;88-B:351–357.
24. Patzakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. *Clin Orthop Relat Res* 1989;243:36–40.
25. MacKenzie EJ, Bosse MJ, Pollak AN, et al. Long-term persistence of disability following severe lower-limb trauma. Results of a seven-year follow-up. *J Bone Joint Surg [Am]* 2005;87-A:1801–1809.

■ TRAUMA

Severe open tibial fractures in combat trauma

MANAGEMENT AND PRELIMINARY OUTCOMES

J. G. Penn-Barwell,
P. M. Bennett,
C. A. Fries,
J. M. Kendrew,
M. J. Midwinter,
R. F. Rickard

*From The Royal
Centre for Defence
Medicine,
Birmingham, United
Kingdom*

■ J. G. Penn-Barwell, MBChB,
MRCS, Trauma and Orthopaedic
Registrar, Research Fellow
■ M. J. Midwinter, CBE, MD,
FRCS(Gen), Defence Professor
of Surgery, Consultant General
Surgeon
Royal Centre for Defence
Medicine, National Institute of
Health Research, Surgical
Reconstruction and
Microbiology Research Centre
(NIHR SRMRC), Queen Elizabeth
Medical Centre, Edgbaston,
Birmingham B15 2TH, UK.

■ P. M. Bennett, MBChB,
Medical Officer
■ J. M. Kendrew, FRCS(Tr &
Orth), Trauma and Orthopaedic
Consultant
Royal Centre for Defence
Medicine, Queen Elizabeth
Medical Centre, Edgbaston,
Birmingham B15 2TH, UK.

■ C. A. Fries, MBChB, MRCS,
Burns and Plastic Surgery
Registrar, Research Fellow
■ R. F. Rickard, PhD, FRCS,
FRCSEd(Plast), Senior Lecturer
in Surgery, Consultant Plastic
Surgeon
Royal Centre for Defence
Medicine, Plymouth Hospitals
NHS Trust, Derriford Road,
Crownhill, Plymouth, Devon,
PL6 8DH, UK.

Correspondence should be sent
to Surg Lt Cdr J. G. Penn-Barwell
RN; e-mail:
jowan@doctors.net.uk

©2013 British Editorial Society
of Bone & Joint Surgery
doi:10.1302/0301-620X.95B1.
30580 \$2.00

Bone Joint J
2013;95-B:101-5.
Received 1 August 2012;
Accepted 10 October 2012

The aim of this study was to report the pattern of severe open diaphyseal tibial fractures sustained by military personnel, and their orthopaedic-plastic surgical management. The United Kingdom Military Trauma Registry was searched for all such fractures sustained between 2006 and 2010. Data were gathered on demographics, injury, management and preliminary outcome, with 49 patients with 57 severe open tibial fractures identified for in-depth study. The median total number of orthopaedic and plastic surgical procedures per limb was three (2 to 8). Follow-up for 12 months was complete in 52 tibiae (91%), and half the fractures (n = 26) either had united or in the opinion of the treating surgeon were progressing towards union. The relationship between healing without further intervention was examined for multiple variables. Neither the New Injury Severity Score, the method of internal fixation, the requirement for vascularised soft-tissue cover nor the degree of bone loss was associated with poor bony healing. Infection occurred in 12 of 52 tibiae (23%) and was associated with poor bony healing (p = 0.008). This series characterises the complex orthopaedic-plastic surgical management of severe open tibial fractures sustained in combat and defines the importance of aggressive prevention of infection.

Cite this article: *Bone Joint J* 2013;95-B:101-5.

Injuries from modern warfare mainly affect the limbs.¹ It is likely that improvements in body armour and helmets, coupled with the use of improvised explosive devices (IEDs), have led to an increase in the severity of limb injuries in those who would have previously suffered fatal injuries to the chest and abdomen. The superficial nature of the tibia and its poor blood supply make the management of open tibial fractures difficult.² Evidence from civilian practice shows that combined management of these injuries in specialist centres, adopted as the standard of care by the British Orthopaedic Association and the British Association of Plastic, Reconstructive and Aesthetic Surgeons,³ improves outcomes.⁴⁻⁶

Open fractures of the tibia sustained during combat differ from those usually seen in the civilian setting. The energy transferred from military munitions and IEDs is considerably higher than that caused by road traffic accidents, falls or civilian gunshot wounds. This difference manifests most clearly in the degree of tissue loss and periosteal stripping that commonly occurs in combat injuries. Bone and soft tissues are either blasted from the limb at the moment of injury or are devitalised, requiring surgical excision. Severe contamination at the time of injury is almost universal and requires

extensive debridement as part of the surgical prevention of infection.

The aim of this study was to characterise the pattern of severe open tibial fractures encountered in current conflicts and to identify factors that might be associated with a poor early outcome.

Patients and Methods

The United Kingdom Military Joint Theatre Trauma Registry (JTTR) captures data on all patients admitted to British military medical facilities who are subsequently repatriated for treatment. We searched for codes that included bony injuries of the knee, tibia or ankle sustained between 1 April 2006 and 30 September 2010 in United Kingdom personnel. The clinical records and radiographs of these patients were reviewed and the following were excluded: injuries not involving the tibial diaphysis (AO/Müller type 42⁷); injuries other than those graded by the operating surgeon as Gustilo-Anderson grade III⁸; and patients who were managed with a primary amputation (either below- or above-knee) within the first three surgical episodes.

Data were gathered on the patients' demographics, injury and surgical management. Outcomes were determined at 12 months and

Table I. Grading system for bone loss¹⁰

Grade	Definition
0	None
1	Minimal, some bone loss but < 1 cm longitudinally around > 50% of the circumference of the shaft and with some cortical contact
2	Moderate, bone loss between 1 and 2 cm around > 50% of the circumference of the shaft but with some cortical contact
3	Severe, bone loss > 2 cm around > 50% of the circumference of the shaft but with some cortical contact
4	Segmental bone loss

were regarded as good if the fracture had united or, in the opinion of the treating surgeon, was clinically and radiologically progressing towards union. Unplanned revision of fixation or bone grafting were used as surrogate markers of a lack of healing and, together with amputation after the third surgical episode, were regarded as poor outcomes.

The following factors were analysed: 1) New Injury Severity Score (NISS)⁹; 2) degree of tibial bone loss, which was graded according to a modification of Robinson et al's¹⁰ classification (Table I); 3) fixation, which was dichotomised into internal (intramedullary (IM) nail or plates and screws) *versus* external fixation (monoaxial and circular frames) or splintage; 4) soft tissues (the requirement for vascularised soft-tissue reconstruction was recorded, and when more than one plastic surgical technique was used, the most complicated was recorded); 5) infection, with a limb deemed to have been infected if surgical treatment for infection was undertaken.

Statistical analysis. Descriptive data are given as means with standard deviations (SD), or medians with ranges where appropriate. Continuous data were analysed using Wilcoxon's rank sum test with continuity correction. The other four factors were analysed using Fisher's exact test. In interpreting the levels of statistical significance, a Bonferroni correction was applied to avoid the increased risk of a type 1 error inherent in multiple comparisons. In order to allow for the five comparisons, significance was set at a p-value < 0.01.

Results

The search of the JTTR database elicited 233 patients with bony injuries affecting the knee, tibia or ankle. After review of case notes and radiographs, 184 were excluded according to the predefined criteria, leaving 49 patients with 57 severe open tibial fractures. The mean age of these patients was 26 years (19 to 43). Most patients (n = 35, 71%) had sustained injuries from a blast (Table II). The grading of the 57 fractures according to the Gustilo-Anderson classification is shown in Table III. A total of 15 fractures (26%) were in patients who had an associated fracture of the ipsilateral foot, and four (7%) had an ipsilateral femoral fracture. A total of eight fractures (14%) had an associated nerve injury and seven patients (14%) had suffered a traumatic amputation of the contralateral leg. There were contralateral fractures of the

Table II. Mechanism of injury in the 49 patients

Mechanism	n (%)
Blast – improvised explosive device	31 (63)
Blast – indirect fire (e.g. mortars, rockets)	4 (8)
Gunshot wounds	13 (27)
Other	1 (2)

Table III. Gustilo-Anderson classification of the 57 fractures according to the surgeon at the initial procedure

Gustilo-Anderson classification	n (%)
3A	10 (17)
3B	43 (75)
3C	4 (7)

calcanei in four patients (7%), of the tibia (closed) in three (5%) and of the femur in four (7%).

A total of 27 limbs (47%) required immediate four-compartment fasciotomies; 40 (70%) were initially stabilised with an external fixator. The remaining 17 (30%) were splinted with Plaster of Paris. The median number of orthopaedic and plastic surgical procedures per limb was three (two to eight).

Outcomes. In five patients (five tibiae) insufficient information could be retrieved for the outcome at 12 months to be determined. Complete follow-up data were therefore available for 44 patients (52 tibiae; 91%). At 12 months a total of 26 (50%) had achieved union, or were progressing clinically and radiologically towards union. Revision surgery had been required for 19 fractures (36%) because of either a failure of fixation or lack of progress towards union. Amputation had been required for seven fractures (13%), owing to ischaemia in one, infection in three, pain or deformity in one and an unsalvageable calcaneal fracture in two. In all instances it was felt that further attempts at salvage would be inappropriate.

The median NISS was 22 (mean 25.1 (4 to 45)), reflecting the severe injuries in these patients. The median score in the group with a good outcome was 19.5 (interquartile range (IQR) 10 to 26.75) and in those with a bad outcome it was 22 (IQR 17 to 27). There was no statistically significant difference in median scores between the two groups (p = 0.179, Wilcoxon's rank sum test) (Fig. 1).

The degree of bone loss after debridement is shown in Table IV. Bone grafting was used in five fractures (9.6%). Bone loss scores showed no statistically significant difference (p = 0.046, Fisher's exact test) (Fig. 2).

Definitive fixation was achieved using an intramedullary nail in 29 fractures (51%) and using plates and screws in 14 (25%). Further breakdown of the management of the fractures is shown in Table V. The median delay to fixation was three days (1 to 51) from the time of injury. No relationship was found between the method of fixation and outcome (p = 1.0, Fisher's exact test).

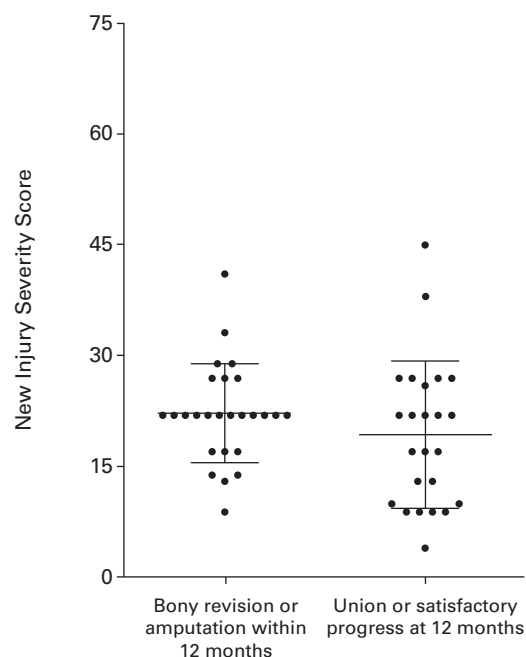


Fig. 1

Scatter plot showing the New Injury Severity Score by 'poor' and 'good' initial bony-healing results. The horizontal lines denote the mean and the standard deviation.

Table IV. Grade of bone loss

Grade	n (%)
0	25 (44)
1	21 (37)
2	1 (2)
3	10 (17)
4	0

The most common technique used for initial soft-tissue cover was split skin grafting (22 fractures, 39%). In 18 (32%) it was thought that the pattern of soft-tissue and bony injury necessitated flap coverage (Table VI). No statistically significant association was found between the need for a flap and the outcome ($p = 0.538$, Fisher's exact test).

Deep infection requiring surgical treatment occurred during the management of 12 of 52 fractures (23%); 11 occurred in limbs with a poor outcome. There was a statistically significant association between deep infection requiring surgical treatment and poor outcome ($p = 0.008$, Fisher's exact test).

Discussion

This study characterises the challenging nature of the management of severe open tibial fractures sustained in combat. A typical case is illustrated in Figures 3 to 5. The rate of infection was 23%. Analysis suggests that infection requiring surgical treatment is significantly associated with impaired bony healing. Neither NISS, severity of bone loss,

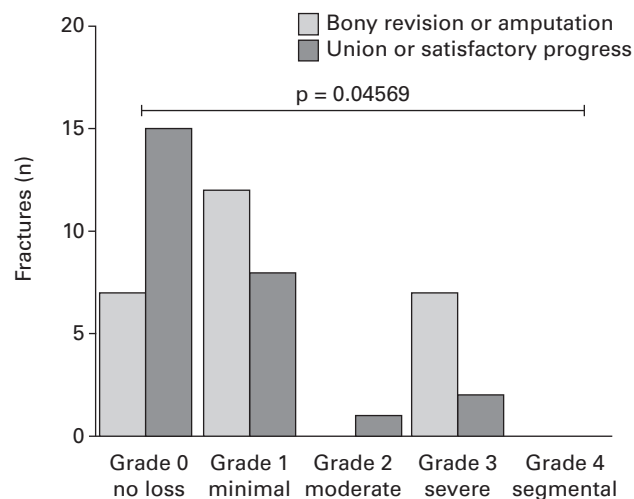


Fig. 2

Bar chart showing bone loss and outcome at 12 months.

Table V. The technique of initial fixation

Management	n (%)
Intramedullary nails	29 (51)
Unreamed	10
Reamed	19
Plates	14 (25)
Monoaxial external fixation	6 (11)
Circular external fixation	2 (4)
Conservative	6 (11)

Table VI. Plastic surgical techniques used for initial wound cover

Technique	n (%)
Secondary intention	3 (5)
Delayed primary closure	8 (14)
Split skin graft	22 (39)
Pedicled flap	10 (18)
Free flap	8 (14)
No data	6 (11)

type of fixation, nor the need for vascularised soft-tissue reconstruction was associated with outcome at 12 months.

There are clearly weaknesses in this retrospective observational study. There may be additional confounding factors within such a heterogeneous population treated by different surgeons. The outcome measures used are clinically relevant but lack objectivity.

Many surgeons would regard amputation later than the first or second procedure as a failure of salvage. However, owing to the unique constraints inherent in the care of combat casualties, amputation was regarded in this study as a failure only if it was required after three procedures. This reflects the practice of temporarily stabilising some limbs of dubious functional viability prior to transporting the patient back to the United Kingdom.

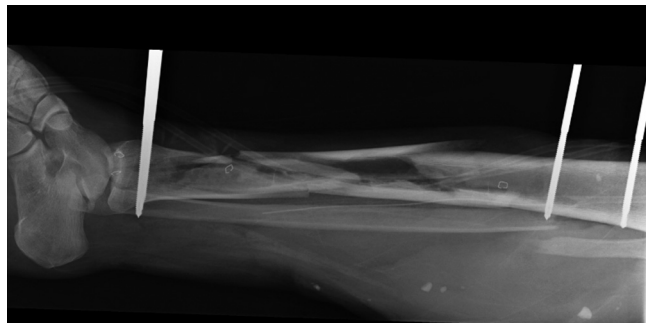


Fig. 3a



Fig. 3b

Radiograph (a) and clinical photograph (b) showing the appearance of a typical blast open tibial fracture at first surgical episode following repatriation to the United Kingdom, on the second day after injury. The initial surgical treatment on the day of injury comprised debridement, external fixation and the application of topical negative-pressure dressings.

Current strategies from the time of injury to initial surgery include damage control resuscitation,¹¹ and are directed to saving life. The first surgical episode is focused on restoring perfusion and reducing contamination. Contemporary basic science and clinical evidence support the necessity of restoring limb perfusion as soon as possible to optimise neuromuscular functional recovery.¹² Recent evidence suggests that haemorrhagic shock can exacerbate the effect of ischaemia within as little as one hour, with three hours of ischaemia causing irreversible functional deficit.¹³ Neuromuscular damage may mean that limb salvage does not lead to a good outcome, even if fracture union is achieved. Newer resuscitation strategies, which include the use of adjuncts such as statins, ethyl pyruvate or limb hypothermia, may be required in the future, not only to save life but also to promote functional recovery.^{14,15}

During the first surgical episode after repatriation to the United Kingdom, the priorities are not only further removal of contamination but also evaluation of the degree of tissue viability and planning definitive treatment. At both of these operations a limb that is perfused but regarded by the treating surgeon as unreconstructable, might not be amputated, as it might be a source of vascularised tissue, e.g. foot fillet flaps, and graft material (skin, bone and nerve) that could be used to maintain limb



Fig. 4

Intra-operative clinical photograph. On the third day the external fixator was removed, an unreamed nail inserted and the defect covered with free semitendinosus flap from the contralateral leg (performed by DE and TC) and a local gastrocnemius flap. This photograph was taken on day nine as muscle flaps are covered with split skin grafts.

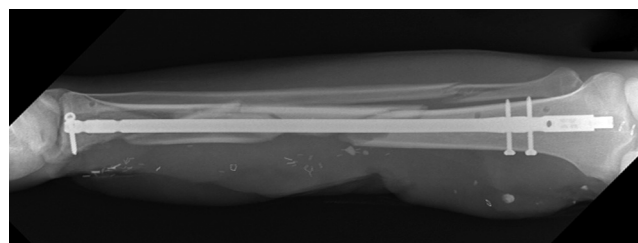


Fig. 5

Plain anteroposterior radiograph showing the nail *in situ* and the degree of bone loss (grade 3 bone loss > 2 cm around at least 50% of the circumference of the bone but with some cortical contact).

length or reconstruct other injuries. Typically, the definitive procedure is the third surgical episode.

In this study a 12-month follow-up was used, as although the acute care of casualties takes place at one institution, these patients come from all regions of the United Kingdom and at their request ongoing care may be transferred to a hospital closer to their home. Therefore, after 12 months there is a significant drop in the number of patients being followed up. It is likely that if the follow-up period was longer, a greater proportion of poor outcomes would be detected. However, the authors regard the 91% follow-up of fractures as sufficient for conclusions to be drawn and is comparable to similar published work.¹⁶

Infection is notoriously difficult to define in patients with combat injuries. All combat wounds lie somewhere on the spectrum of contamination–colonisation–infection. Both surgical and pharmacological treatments are used aggressively in all cases to prevent and treat infection. A requirement for further surgical debridement was a definition of infection.

The point at which a bone defect becomes critical, i.e. one that will not heal, is poorly understood in the clinical setting, as it will depend on both the anatomical site of the defect, its surrounding soft-tissue envelope and the patient's comorbidities.

In 1995 Robinson et al¹⁰ suggested that loss of > 2.5 cm involving > 50% of the circumference of the tibia constituted

a critical defect, as ten out of 11 of these fractures required grafting or revision surgery. A sub-analysis of the Study to Prospectively Evaluate Reamed Intramedullary Nails in Tibial Fractures (SPRINT) found that of half the defects > 1 cm, over > 50% of the circumference healed without further surgical intervention after nailing.¹⁷ Therefore, for the purposes of this study, instead of a single definition of a critical defect a continuous grading system was used, based on a simplified version of that proposed by Robinson et al.¹⁰

Revision surgery (as well as amputation) was used by the authors as a marker of treatment failure, based on the finding of the Lower Extremity Assessment Project (LEAP) that re-hospitalisation was closely associated with a poor outcome¹⁸ and the evidence that late amputation is associated with a worse outcome than both limb salvage and primary amputation.¹⁹ It is recognised that the debate over the choice between limb salvage and amputation is unresolved. Long-term studies in military patients comparing modern prostheses with salvaged limbs are not yet available.

Open tibial fractures sustained in combat differ from those sustained in the civilian setting: the overall transfer of energy is greater, resulting in larger zones of injury, with the corollary that bony reconstruction with soft-tissue cover can be difficult to achieve. Infection is significantly associated with impaired bone healing. However, the limb can still be salvaged using a combined orthopaedic-plastic surgical approach.

On behalf of the Severe Lower Extremity Combat Trauma (SeLECT) Study Group: Surg Lt Cdr J. G. Penn-Barwell, Surg Lt P. M. Bennett, Surg Lt Cdr C. A. Fries, Wg Cdr J. M. Kendrew, Surg Capt M. Midwinter, Surg Cdr R. F. Rickard, Gp Capt I. D. Sargeant, Professor K. Porter, Lt Col T. Rowlands, Lt Col A. Mountain, Miss D. Bose, Lt Col S. Jeffery, Wg Cdr D. Evriviades, Wg Cdr W. J. C. van Niekerk, Col A. Kay L/RAMC, Lt Col T. Cubison, Mr D. Wallace, Mr D. Power, Mr J. Cooper, Royal Centre for Defence Medicine/Queen Elizabeth's Hospital, Birmingham, United Kingdom.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Ministry of Defence or Her Majesty's Government. None of the authors have any conflicts of interest with the subject of this study. All of the authors are serving officers in the Royal Navy or Royal Air Force and the copyright of this work remains with the crown.

The Academic Department of Military Emergency Medicine (ADMED), Defence Analytical Services and Advice (DASA) are thanked for collecting, collating and identifying the appropriate data for this paper. The professionalism of the Medical, Nursing and support staff of the Defence Medical Services and University Hospitals Birmingham for their work in treating the patients described in this study is gratefully acknowledged.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

1. Owens BD, Kragh JF Jr, Macaitis J, Svoboda SJ, Wenke JC. Characterization of extremity wounds in Operation Iraqi Freedom and Operation Enduring Freedom. *J Orthop Trauma* 2007;21:254–257.
2. Patzakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. *Clin Orthop Relat Res* 1989;243:36–40.
3. Nanchahal J, Nayagam S, Khan U, et al. Standards for the management of open fractures of the lower limb. <http://www.bapras.org.uk/downloaddoc.asp?id=379> (date last accessed 11 October 2012).
4. Hertel R, Lambert SM, Müller S, Ballmer FT, Ganz R. On the timing of soft-tissue reconstruction for open fractures of the lower leg. *Arch Orthop Trauma Surg* 1999;119:7–12.
5. Okike K, Bhattacharyya T. Trends in the management of open fractures: a critical analysis. *J Bone Joint Surg [Am]* 2006;88-A:2739–2748.
6. Naique SB, Pearse M, Nanchahal J. Management of severe open tibial fractures: the need for combined orthopaedic and plastic surgical treatment in specialist centres. *J Bone Joint Surg [Br]* 2006;88-B:351–357.
7. Marsh JL, Slongo TF, Agel J, et al. Fracture and dislocation classification compendium, 2007: Orthopaedic Trauma Association, database and outcomes committee. *J Orthop Trauma* 2007;21(Suppl):S1–133.
8. Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. *J Bone Joint Surg [Am]* 1976;58-A:453–458.
9. Osler T, Baker SP, Long WB. A modification of the injury severity score that both improves accuracy and simplifies scoring. *J Trauma* 1997;43:922–926.
10. Robinson CM, McLauchlan G, Christie J, McQueen MM, Court-Brown CM. Tibial fractures with bone loss treated by primary reamed intramedullary nailing. *J Bone Joint Surg [Br]* 1995;77-B:906–913.
11. Mahoney PF, Hodgetts TJ, Midwinter M, Russell R. The combat casualty care special edition. *J R Army Med Corps* 2007;153:235–236.
12. Glass GE, Pearse MF, Nanchahal J. Improving lower limb salvage following fractures with vascular injury: a systematic review and new management algorithm. *J Plast Reconstr Aesthet Surg* 2009;62:571–579.
13. Percival TJ, Rasmussen TE. Reperfusion strategies in the management of extremity vascular injury with ischaemia. *Br J Surg* 2012;99(Suppl):66–74.
14. Efron DT, Sorock G, Haut ER, et al. Preinjury statin use is associated with improved in-hospital survival in elderly trauma patients. *J Trauma* 2008;64:66–74.
15. Dong W, Cai B, Peña G, et al. Ethyl pyruvate prevents inflammatory responses and organ damage during resuscitation in porcine hemorrhage. *Shock* 2010;34:205–213.
16. Zelle BA, Bhandari M, Sanchez AI, et al. Loss of follow-up in orthopaedic outcome studies: is 80% follow-up still acceptable? Presented at the Orthopaedic Trauma Association OTA Annual Meeting 2011.
17. Sanders DW. The critical-sized defect in the tibia: is it critical? Results from the SPRINT Trial. Paper presented at the 2010 Annual Meeting of the Orthopaedic Trauma Association. Baltimore, 2010.
18. Bosse MJ, MacKenzie EJ, Kellam JF, et al. An analysis of outcomes of reconstruction or amputation after leg-threatening injuries. *N Engl J Med* 2002;347:1924–1931.
19. Dagum AB, Best AK, Schemitsch EH, et al. Salvage after severe lower-extremity trauma: are the outcomes worth the means? *Plast Reconstr Surg* 1999;103:1212–1220.

Salvage of Combat Hindfoot Fractures in 2003-2014 UK Military

Foot & Ankle International®

1-7

© The Author(s) 2017

Reprints and permissions:

sagepub.com/journalsPermissions.nav

DOI: 10.1177/1071100717697913

journals.sagepub.com/home/fai

Philippa M. Bennett, MBChB, MRCS¹, Thomas Stevenson, MBBS, MRCS¹,
Ian D. Sargeant, MBBS, FRCS², Alistair Mountain, MBChB, FRCS²,
and Jowan G. Penn-Barwell, MBChB, FRCS^{1,3}

Abstract

Background: Hindfoot fractures pose a considerable challenge to military orthopaedic surgeons, as combat injuries are typically the result of energy transfers not seen in civilian practice. This study aimed to characterize the pattern of hindfoot injuries sustained by UK military casualties in recent conflicts, define the early amputation and infection rate, and identify factors associated with poor early outcomes.

Methods: The UK Joint Theatre Trauma Registry was searched for British military casualties sustaining a hindfoot fracture from Iraq and Afghanistan between 2003 and 2014. Data on the injury pattern and management were obtained along with 18-month follow-up data. Statistical analysis was performed with the chi-square test and binomial logistic regression analysis. The threshold for significance was set at $P < .05$. One hundred fourteen patients sustained 134 hindfoot injuries. Eighteen-month follow-up was available for 92 patients (81%) and 114 hindfeet (85%).

Results: The calcaneus was fractured in 116 cases (87%); 54 (47%) were managed conservatively, 32 (28%) underwent K-wire fixation, and 30 (26%) underwent internal fixation. Nineteen patients (17%) required transtibial amputation during this time. A deep infection requiring operative treatment occurred in 13 cases (11%) with *Staphylococcus aureus*, the most common infectious organism (46%). A deep infection was strongly associated with operative fracture management ($P = .0016$). When controlling for multiple variables, the presence of a deep infection was significantly associated with a requirement for amputation at 18 months ($P = .023$). There was no association between open fractures and a requirement for amputation at 18 months ($P = .640$), nor was conservative management associated with a requirement for amputation ($P = .999$). Thirty-six fractures (32%) required unplanned revision surgery within the first 18 months following salvage, of which 19 (53%) involved amputation.

Conclusion: A deep infection was the sole variable significantly associated with a requirement for amputation by 18 months. These results suggest that attempts at salvaging these injuries are at the limits of orthopaedic technical feasibility.

Level of Evidence: Level III, comparative series.

Keywords: war, combat, injuries and wounds, limb salvage, hindfoot, calcaneus, talus, outcomes

Injuries of the lower limbs have been the predominant injury pattern of the recent conflicts in Iraq and Afghanistan.²² Hindfoot and ankle fractures have emerged from these conflicts as the greatest challenge to the orthopaedic surgeon trying to salvage a traumatized limb and provide the patient with pain-free function.^{7,24} In military patients, hindfoot injuries caused by blasts from improvised explosive devices (IEDs) are highly complex.²⁴ In the civilian setting, the optimal management of intra-articular calcaneal fractures remains controversial.^{10,21} Various treatment strategies have been employed to manage these injuries in military patients, and so far, no systematic examination of outcomes has been performed. As a result, there is a lack of consensus on how best to manage this combat injury pattern and uncertainty about patients' outcomes.

When considering the issue of limb salvage, there are 2 questions to be answered. First, *could* an injured limb be

¹Institute of Naval Medicine, Alverstoke, UK

²Royal Centre for Defence Medicine, Queen Elizabeth Hospital, Edgbaston, UK

³Surgical Reconstruction and Microbiology Research Centre, National Institute for Health Research, Birmingham, UK

On behalf of the Severe Lower Extremity Combat Trauma (SeLECT) Study Group: Surg Lt Cdr J.G. Penn-Barwell, Surg Lt Cdr P.M. Bennett, Surg Lt Cdr C.A. Fries, Gp Capt J.M. Kendrew, Surg Capt M. Midwinter, Dr J. Bishop, Surg Capt R.F. Rickard, Gp Capt I.D. Sargeant OBE, Prof Sir K. Porter KBE, Lt Col T. Rowlands, Lt Col A. Mountain, Miss D. Bose, Lt Col S. Jeffrey, Dr D. Mortiboy, Mr A.F.G. Groom, Surg Lt R.W. Myatt, Surg Lt Cdr T. Stevenson, Surg Capt S.A. Stapley, Lt Col M. Foster, and Col A. Kay.

Corresponding Author:

Philippa M. Bennett, MBChB, MRCS, Institute of Naval Medicine, Crescent Road, Alverstoke, Hampshire, PO12 2DL, UK
Email: pippa_bennett@icloud.com

salvaged? Second, *should* it be? The first question aims to address whether it is technically possible to salvage a limb, free from infections and with all fractures and wounds healed. The second question assesses whether a patient would be better off in the long term with a salvaged limb or with amputation. For the study period examined, the assumption of the treating operative team was that hindfoot injuries should be salvaged if at all feasible. This approach aimed not only to retain a limb that may provide improved function over a prosthesis for the patient over the course of his or her life but also to retain the choice of delayed amputation for the patient in the future. The aim of this study was therefore to characterize the pattern of hindfoot injuries sustained by UK military casualties in recent conflicts, define the early amputation and infection rate, and identify factors associated with poor early outcomes.

Methods

The study was registered with, and approved by, the Joint Medical Command. The UK Joint Theatre Trauma Registry (JTTR) is an electronic database administered by Defence Statistics that prospectively captured data on all casualties sustained overseas.¹² Data were gathered on casualties either killed immediately or who were injured and triggered a trauma alert at a deployed medical facility. Data on casualties whose injuries subsequently required them to be repatriated to the UK were also collected. Injuries were coded according to the 2005 military version of the Abbreviated Injury Scale (AIS).¹ Data were gathered by research nurses at deployed medical facilities and at the Royal Centre for Defence Medicine (RCDM) in Edgbaston, United Kingdom.¹²

The JTTR was searched for AIS codes that described bony injuries of the foot, ankle, fibula, tibia, calcaneus, and other tarsal bones sustained between January 2003 and December 2014. Only UK survivors were included. Clinical notes and radiographs were reviewed. All fractures of the calcaneus and talus were included for analysis, whether they were intra-articular or extra-articular. In patients sustaining a fracture of the calcaneus or talus, coexisting fractures of the ipsilateral tibial plafond, midfoot, and metatarsals were noted. No stratification was performed on the severity of the talus or calcaneus injury. Patients who were managed with primary amputation within the first 3 operative episodes were excluded. There were 3043 casualties injured within the JTTR in the study period; 555 patients met the initial inclusion criteria, and 441 were excluded according to the predefined criteria. This left 114 (114/3043, 3.7%) patients with 134 injured hindfeet for inclusion in the study; 20 patients sustained bilateral fractures. The median age of the patients was 26 years (interquartile range [IQR], 21-28), and all but 1 were male (113/114, 99%). Eighteen-month follow-up was available for 92 patients (92/114, 81%) and 114 hindfeet (114/134, 85%).

Table 1. Mechanism of Injury.

Mechanism	n (%)
Gunshot wound	10 (9)
Explosive: improvised explosive device	96 (84)
Explosive: indirect fire from weapon (eg, mortar)	5 (4)
Other	3 (3)

Data were gathered on patients' demographics, injury, operative management, and New Injury Severity Score (NISS). Unlike the Injury Severity Score in which only the most severe injury per body region is included, the NISS considers the 3 most severe injuries regardless of the body region.¹⁹ The total is calculated as the sum of the squares of the 3 most severe AIS injuries for a maximum of 75. Patients' hospital records were reviewed along with the Ministry of Defence primary care electronic record: the Defence Medical Information Capability Programme. A deep infection was defined as that requiring operative debridement; the causative organism was identified by an interpretation of deep culture samples by a consultant microbiologist.¹³ Removal of symptomatic hardware was considered to be a revision procedure.

Statistical Analysis

Descriptive data are presented as the median with IQR. Statistical analysis was performed with the use of SPSS v23 software (IBM, Armonk, NY, USA). The chi-square test was used to examine the relationship between dichotomous variables. Variables with the strongest associations were used to develop a binomial logistic regression model. This identified variables associated with a requirement for amputation at 18 months while controlling for potential bias. The threshold for significance was set at .05.

Results

The majority (101/114, 89%) were injured by explosive weapons, mainly IEDs, with further detail given in Table 1. The median NISS¹⁹ was 12 (IQR, 8-17), with nearly a third of patients (34/114, 30%) having an NISS above 15, the commonly used definition of major trauma.⁴ Two patients had the maximum NISS of 75, regarded as indicative of an unsurvivable injury burden.

The calcaneus was the most frequently injured bone, being fractured in 116 cases (116/134, 87%), which occurred in isolation in 64 cases (64/134, 48%). Thirty-eight patients sustained a fracture of the talus (38/134, 28%), with further detail given in Table 2.

Three cases (3/134, 2%) sustained an associated arterial injury that required repair; 9 (9/134, 7%) sustained an associated nerve injury. Thirty-three cases (33/134, 25%)

Table 2. Injury Pattern by Bone.

Fracture	Total, n (%)	Isolated, n (%)
Calcaneus	116 (87)	64 (48)
Talus	38 (28)	11 (8)
Coexisting tibial plafond	33 (25)	—
Coexisting other tarsal	61 (46)	—
Coexisting metatarsal	53 (40)	—

Patients with isolated fractures of the tibial plafond, tarsals, and metatarsals were not included in the analysis.

Table 3. Initial Fracture Management.

Management	Talus (n = 38), n (%)		Calcaneus (n = 116), n (%)	
	Closed	Open	Closed	Open
Nonoperative	15 (39)	7 (18)	35 (30)	19 (16)
External fixation	—	5 (13)	—	—
K-wire	4 (10)	1 (2)	19 (16)	13 (11)
Internal fixation	3 (8)	3 (8)	20 (17)	10 (9)

sustained an ipsilateral fracture proximal to the ankle; 8 (8/114, 7%) patients suffered traumatic contralateral amputation at or proximal to the transtibial level.

Fifty cases (50/134, 37%) were open fractures. Military guidelines stated that all blast or ballistic trauma patients should receive antibiotics within 1 hour of wounding, typically 1.2 g of benzylpenicillin administered by combat medical technicians. Once at a formal medical facility, patients were given further antibiotics, either co-amoxiclav or ceftriaxone and metronidazole.²⁸ These were continued while the patient was repatriated to the RCDM, where they were tailored based on microbiological results and continued based on usual clinical parameters. The most common method of soft tissue coverage or closure was secondary intention, typically after the use of topical negative pressure dressings (28/50, 56%). Pedicled flaps were used in 3 cases (3/50, 6%), with 7 cases requiring a free flap (7/50, 14%).

Of the 116 calcaneal fractures, 54 were managed nonoperatively (54/116, 47%), with the remaining 62 undergoing internal fixation (62/116, 53%) either with percutaneous K-wires or formal open reduction internal fixation. Further detail on fracture management is shown in Table 3. The median delay to definitive fixation was 2 days (IQR, 0-8), and the median number of operative procedures was 1 (IQR, 1-1).

Follow-up

Thirty-six (36/114, 31%) required unplanned revision surgery on their injured limb within the first 18 months. Nineteen

Table 4. Indications for Revision Surgery for 114 Injured Hindfeet With 18-Month Follow-up Data Available.

Procedure and Indication	n (%)
Amputation	19 (17)
Aseptic nonunion/avascular necrosis	6 (5)
Deep infection	6 (5)
Pain despite radiographic union	5 (4)
Nonviable soft tissues	2 (2)
Revision other than amputation	17 (15)
Washout/debridement for deep infection	7 (6)
Arthrodesis	6 (5)
Osteophyte removal	3 (3)
Revision of metal construct	1 (1)

Table 5. Causative Organisms in the 13 Cases of Infection.

Organism	n (%)
<i>Staphylococcus aureus</i>	6 (46)
<i>Pseudomonas</i>	2 (15)
<i>Escherichia coli</i>	1 (8)
Cultures not available	3
No growth	1

limbs (19/114, 17%) required transtibial amputation: 6 were performed for nonunion, with further detail on indications given in Table 4. The remaining 17 cases (17/114, 15%) required a revision procedure other than amputation at a median of 10 months (IQR, 8-12), with further detail given in Table 4. Five patients (5/54, 9%) whose calcaneal fractures were managed nonoperatively underwent amputation within 18 months: 2 of these were for a deep infection in patients with open fractures, 2 for nonunion, and 1 for pain despite radiographic union. There was no statistical association between a requirement for revision surgery and whether the initial injury was open or closed ($P = .071$).

A deep infection, defined as requiring operative debridement, occurred in 13 cases (13/114, 11%) in the first 18 months.¹³ There were no deep infections in closed fractures managed nonoperatively. Four closed fractures managed operatively (1 with K-wires, 3 with open reduction internal fixation) developed a deep infection postoperatively. Nine open fractures developed a deep infection: 1 of these was nonoperatively managed, 4 were fixed with K-wires, and 4 were repaired with open reduction internal fixation. Operative fracture management was significantly associated with the development of a deep infection ($P = .0016$). The median duration for the development of a deep infection was 2 months (IQR, 1-10), although 1 infection did not become clinically apparent until 13 months after the injury. The most common infectious organism was *Staphylococcus aureus*, with further detail given in Table 5. All 13 patients who developed a deep infection required a revision procedure ($P = .0001$).

Table 6. Binomial Logistic Regression Analysis to Examine Variables Associated With a Requirement for Amputation at 18 Months.

Variable	Logit Coefficient β	Odds Ratio (95% Confidence Interval)	P Value
Open fracture	-0.309	0.201-2.686	.640
Deep infection	-1.918	0.028-0.770	.023
Nonoperative management	0.001	0.118-8.506	.999
K-wire fixation	-1.167	0.040-2.412	.264
Open reduction internal fixation	0.486	0.148-17.879	.691
Coexisting tibial plafond fracture	20.083	—	.998
Coexisting midfoot fracture	-1.019	0.093-1.402	.141

R^2 coefficient = 0.362.

Of the cases requiring soft tissue coverage, the use of vascularized tissue transfer was not associated with the development of a deep infection ($P = .6639$) or amputation by 18 months ($P = .3689$). Only 1 of the 7 free flaps failed, which occurred at 19 days after surgery and was revised but eventually required transtibial amputation 14 months after the injury.

A binomial logistic regression model was developed to analyze the requirement for amputation at 18 months while controlling for multiple variables. The results of Table 6 show that a deep infection alone was associated with a significant requirement for amputation at 18 months ($P = .023$). Open fractures were not associated with a greater requirement for amputation at 18 months ($P = .640$) compared to closed fractures. The choice of conservative management ($P = .999$), K-wire fixation ($P = .264$), or open reduction internal fixation ($P = .691$) was not associated with amputation at 18 months.

Discussion

This study characterizes the hindfoot injuries sustained by UK service personnel in the 12 years of conflict in Iraq and Afghanistan and defines outcomes at 18 months. The deep infection rate in these injuries was 11%, and the overall amputation rate was 17%. While controlling for multiple variables, the presence of a deep infection significantly raised the requirement for amputation by 18 months.

Our study did not show an association between open fractures and a requirement for amputation. A previous study on combat foot and ankle injuries found that open fractures were an independent predictor for the requirement for amputation; however, this included limbs amputated primarily at a field hospital.²⁴ Many surgeons may regard amputation later than the first or second procedure as the failure of salvage rather than the definition used in this study as amputation occurring after 3 procedures. This is a reflection of current UK military operative practice: even if there is considerable doubt over the long-term viability of an injured limb at deployed operative facilities, if it is

perfused, then it is stabilized, and the patient is repatriated to the UK. This recognizes not only the importance of decisions regarding limb salvage being made by a single, experienced operative team but also that the injured limb may be a useful source of vascularized tissue and graft material. Therefore, the third procedure was typically the definitive one.

The relatively low number of patients with open injuries was due to the number of casualties injured when driving over an IED detonation. This is analogous to the “deck slap” injury seen in the Second World War, where mines detonated under ships caused the deck to be accelerated up into the casualty’s foot with great force, although not necessarily causing an open wound.¹⁵ Other studies examining the association between multiple variables and outcomes either confined their analyses solely to open or closed fractures or did not analyze these subgroups further.^{3,5-8,11,14,16,20,26,27,30,32}

The finding that open fractures in this study were not associated with a requirement for amputation supports the authors’ experience, echoed by previously published reports, that the skin encompassing the foot was often in better condition in which fractures were open, effectively decompressing the injury.^{25,29} In cases in which the foot remained closed, the authors frequently observed that massive fracture blisters had developed by 36 hours. The soft tissues inevitably deteriorated from this point, commonly leading to large patches of frank necrosis over the dorsum and lateral side of the foot (Figure 1).²³

Earlier studies noted an increase in postoperative soft tissue problems and inferior results if surgery is delayed beyond 14 days.^{29,31} Previous work in this field has described the severity of injuries seen in hindfoot fractures sustained in combat, with 70% of injuries in the work classified as severe or worse on the Foot and Ankle Severity Scale.^{18,24} It was the authors’ experience that once blisters had developed, it was typically not possible to operate until 6 weeks after the injury due to the gross soft tissue deterioration and, often in this cohort, the generalized, systemic physiological insult. Our strategy therefore evolved to address the foot

injury early, typically at 36 to 72 hours, to prevent soft tissue deterioration; operating after 3 days and before 6 weeks frequently led to wound breakdown and a poor outcome for the patient. This strategy is reflected in the median delay to definitive fixation being only 2 days. The use of K-wires to stabilize fracture fragments avoided the need to raise a soft tissue flap, as would be needed in more invasive fixation techniques, and therefore allowed earlier fixation. Calcaneal fractures that were not amenable to immediate fixation with K-wires were stabilized either with a plaster splint or more commonly with a soft removable splint (Dorsiwedge; DJO Global, Vista, CA, USA). The problem of pin placement precluded the use of external fixators to stabilize these injuries.

Six patients required amputation for aseptic nonunion or avascular necrosis in this study period. As the images in Figure 1 demonstrate, the severe fracture comminution likely rendered some fragments avascular at the time of the injury. Five patients required amputation for pain despite radiographic union. The pain experienced by these patients was likely multifactorial and related to the insult to the nerves sustained at the time of the injury and/or necrosis of the heel fat pad.

Contrary to previous descriptions in the literature, our study did not show an increased risk of deep infections with open fractures compared to closed fractures.^{2,9,25} We report an overall deep infection rate requiring operative intervention of 11%. A direct comparison of our infection rate with that reported in the literature is complicated by the nonstandardized definition of an infection and the variety of fracture patterns reported in this study. All open fractures were treated aggressively with antibiotics; UK military practice is to treat open fractures with 1.2 g of intravenous co-amoxiclav every 8 hours. Patients showing signs of wound sepsis had their antibiotic therapy tailored to microbiological results. In patients without definitive microbiological results, clindamycin was often given in addition to co-amoxiclav, under the guidance of a consultant microbiologist at the multi-disciplinary team (MDT) meeting. Patients injured in aquatic environments such as irrigation channels were also treated with ciprofloxacin to cover for atypical gram negatives, for example, *Aeromonas hydrophila*.

A literature review by Benirschke and Kramer² of calcaneal fractures developing serious infections, that is, those requiring more extensive intervention than the administration of oral antibiotics, cited an infection rate of 0% to 20% for closed fractures and 19% to 31% for open fractures, with their study reporting a serious infection rate of 1.8% in closed fractures and 7.7% in open fractures. We used the definition of a deep infection as that requiring operative treatment, as an infection was difficult to define in combat wounds, which were all recognized as lying somewhere on a spectrum of contamination-colonization-infection.



Figure 1. Clinical images of a patient with bilateral hindfoot fractures. The axial computed tomography slices (A) and reconstructions (B) demonstrate the comminuted nature of the bilateral calcaneal fractures. The clinical images (C) demonstrate the split in the plantar surface of the left-sided injury and the significant swelling frequently seen with closed injuries. The lateral radiograph shows K-wire fixation of the left calcaneal fracture (D), which went on to require subtalar fusion (E).

The follow-up period in this study was limited to 18 months. Following completion of acute treatment in this single, centralized unit, patients' care was often transferred to the Defence Medical Rehabilitation Centre. If they left the military, many opted to be followed in hospitals more local to where they were based to facilitate further appointments. Therefore, there was a substantial drop in the number of patients being followed at our institution after 18 months. Despite this, the authors regard the 81% rate of follow-up of patients and 85% rate of fractures as sufficient to draw initial conclusions. These rates are equivalent to the results of the Lower Extremity Amputation Prevention Project, which cited 86% of follow-up at 12 months and 77% of follow-up at 24 months.¹⁷ Despite this, 1 deep infection in this study did not become apparent until 13 months after the injury, when bony union had already been achieved. The delayed development of a deep infection and other complications has previously been reported, and it is therefore probable that a longer follow-up of this cohort of patients would reveal a higher incidence of deep infections and delayed amputation.^{2,8} At 18 months, we found an amputation rate of 17% and a deep infection rate of 11%. If, in addition to cases included in follow-up, all 20 fractures lost to follow-up required transtibial amputation and developed deep infections, these rates would be 29% and 25%, respectively.

The results of this article demonstrate that the question of whether a limb could, or should, be salvaged remains. Although it is technically possible to salvage a limb, free from infections and with all fractures and wounds healed (the *could*), it is clear that 18 months after the injury, ongoing problems with pain and nonunion cause some patients to opt for amputation (the *should*). However, the potential problems associated with transtibial amputation preclude this being an appropriate treatment strategy for all patients. Although our results are unable to clearly answer which hindfoot fractures would be best managed with primary amputation over salvage, they do provide important new information on the associations between open injuries and deep infections on outcomes. Patient-reported outcome measures will provide further information on whether a limb should have been salvaged, and work is ongoing to collect these data.

The authors recognize that there are weaknesses in a retrospective, observational study of this nature. The heterogeneity of the patients' injuries limited a reliable examination of causation and prevented a direct comparison between groups. It was not possible to evaluate the effect of timing of the administration of antibiotics and subsequent development of deep infections due to the nature of battlefield trauma, and it is possible that variations in antibiotic delivery may have affected our results. Despite these weaknesses, this study describes the orthopaedic burden of

hindfoot injuries sustained by British military casualties in 12 years of combat.

Acknowledgement

We acknowledge the hard work, dedication, and professionalism of all the members of the Defence Medical Services and the National Health Service that cared for the casualties described in this work. We also thank the Clinical Information and Exploitation Team, Joint Medical Command, and UK Defence Statistics (Health) for collecting, collating, and identifying appropriate data for this article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Ministry of Defence of Her Majesty's Government.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. American Association for Automotive Medicine. *The Abbreviated Injury Scale: AIS-80*. Morton Grove, IL: American Association for Automotive Medicine; 1980.
2. Benirschke SK, Kramer PA. Wound healing complications in closed and open calcaneal fractures. *J Orthop Trauma*. 2004;18:1-6.
3. Berry G, Stevens D, Kreder H, et al. Open fractures of the calcaneus: a review of treatment and outcome. *J Orthop Trauma*. 2004;18:202-206.
4. Boyd CR, Tolson MA, Copes WS. Evaluating trauma care: the TRISS method. *J Trauma Acute Care Surg*. 1987;27:370-378.
5. Buckley R, Tough S, McCormack R, et al. Operative compared with nonoperative treatment of displaced intra-articular calcaneal fractures: a prospective, randomized, controlled multicenter trial. *J Bone Joint Surg Am*. 2002;84-A:1733-1744.
6. Csizy M, Buckley R, Tough S, et al. Displaced intra-articular calcaneal fractures: variables predicting late subtalar fusion. *J Orthop Trauma*. 2003;17:106-112.
7. Dickens JF, Kilcoyne KG, Kluk MW, et al. Risk factors for infection and amputation following open, combat-related calcaneal fractures. *J Bone Joint Surg Am*. 2013;95:e24.
8. Ferreira RC, Sakata MA, Costa MT, Frizzo GG, Santin RA. Long-term results of salvage surgery in severely injured feet. *Foot Ankle Int*. 2010;31(2):113-123.
9. Folk JW, Starr AJ, Early JS. Early wound complications of operative treatment of calcaneus fractures: analysis of 190 fractures. *J Orthop Trauma*. 1999;13:369-372.
10. Griffin D, Parsons N, Shaw E, et al. Operative versus non-operative treatment for closed, displaced, intra-articular fractures of the calcaneus: randomised controlled trial. *BMJ*. 2014;349:g4483.

11. Heier KA, Infante AF, Walling AK, Sanders RW. Open fractures of the calcaneus: soft-tissue injury determines outcome. *J Bone Joint Surg Am.* 2003;85:2276-2282.
12. Hodgetts T, Mahoney P, Russell R, Davies S, McLeod J. Trauma governance in the UK defence medical services. *J R Army Med Corps.* 2007;153:239-242.
13. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Am J Infect Control.* 1992;20:271-274.
14. Ibrahim T, Rowsell M, Rennie W, et al. Displaced intra-articular calcaneal fractures: 15-year follow-up of a randomised controlled trial of conservative versus operative treatment. *Injury.* 2007;38:848-855.
15. Keating C. Wounds in naval action. In: Bailey H, ed. *Surgery of Modern Warfare*. 3rd ed. Edinburgh: Williams and Wilkins; 1944:997-1000.
16. Loucks C, Buckley R. Bohler's angle: correlation with outcome in displaced intra-articular calcaneal fractures. *J Orthop Trauma.* 1999;13:554-558.
17. MacKenzie EJ, Bosse MJ, Castillo RC, et al. Functional outcomes following trauma-related lower-extremity amputation. *J Bone Joint Surg Am.* 2004;86:1636-1645.
18. Manoli A, Prasad P, Levine RS. Foot and ankle severity scale (FASS). *Foot Ankle Int.* 1997;18(9):598-602.
19. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *J Trauma Acute Care Surg.* 1997;43:922-926.
20. Paley D, Hall H. Intra-articular fractures of the calcaneus: a critical analysis of results and prognostic factors. *J Bone Joint Surg Am.* 1993;75:342-354.
21. Pearce C, Wong K, Calder J. Calcaneal fractures: selection bias is key. *Bone Joint J.* 2015;97:880-882.
22. Penn-Barwell JG, Roberts SA, Midwinter MJ, Bishop JR. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003–2012. *J Trauma Acute Care Surg.* 2015;78:1014-1020.
23. Poon H, Le Cocq H, Mountain AJ, Sargeant ID. Dermal fenestration with negative pressure wound therapy: a technique for managing soft tissue injuries associated with high-energy complex foot fractures. *J Foot Ankle Surg.* 2016;55:161-165.
24. Ramasamy MA, Hill CAM, Masouros S, et al. Outcomes of IED foot and ankle blast injuries. *J Bone Joint Surg Am.* 2013;95:e25.
25. Rammelt S, Zwipp H. Calcaneus fractures: facts, controversies and recent developments. *Injury.* 2004;35:443-461.
26. Robb C, Deans V, Iqbal M, Cooper J. Comparison of non-operative and surgical treatment of displaced calcaneal fractures. *Foot.* 2007;17:169-173.
27. Rowe CR, Sakellariides HT, Freeman PA, Sorbie C. Fractures of the os calcis: a long-term follow-up study of 146 patients. *JAMA.* 1963;184:920-923.
28. Russell R, Bess A. *JSP 999: Clinical Guidelines for Operations*. London: UK Ministry of Defence; 2012.
29. Sanders R, Fortin P, DiPasquale T, Walling A. Operative treatment in 120 displaced intraarticular calcaneal fractures: results using a prognostic computed tomography scan classification. *Clin Orthop Relat Res.* 1993;290:87-95.
30. Siebert C, Hansen M, Wolter D. Follow-up evaluation of open intra-articular fractures of the calcaneus. *Arch Orthop Trauma Surg.* 1998;117:442-447.
31. Tennent T, Calder P, Salisbury R, Allen P, Eastwood D. The operative management of displaced intra-articular fractures of the calcaneum: a two-centre study using a defined protocol. *Injury.* 2001;32:491-496.
32. Zwipp H, Tscherne H, Thermann H, Weber T. Osteosynthesis of displaced intraarticular fractures of the calcaneus: results in 123 cases. *Clin Orthop Relat Res.* 1993;290:76-86.



■ FOOT AND ANKLE

Outcomes following limb salvage after combat hindfoot injury are inferior to delayed amputation at five years

**P. M. Bennett,
T. Stevenson,
I. D. Sargeant,
A. Mountain,
J. G. Penn-Barwell**

*Institute of Naval
Medicine, Hampshire,
United Kingdom*

Objectives

The surgical challenge with severe hindfoot injuries is one of technical feasibility, and whether the limb can be salvaged. There is an additional question of whether these injuries should be managed with limb salvage, or whether patients would achieve a greater quality of life with a transtibial amputation. This study aims to measure functional outcomes in military patients sustaining hindfoot fractures, and identify injury features associated with poor function.

Methods

Follow-up was attempted in all United Kingdom military casualties sustaining hindfoot fractures. All respondents underwent short-form (SF)-12 scoring; those retaining their limb also completed the American Academy of Orthopaedic Surgeons Foot and Ankle (AAOS F&A) outcomes questionnaire. A multivariate regression analysis identified injury features associated with poor functional recovery.

Results

In 12 years of conflict, 114 patients sustained 134 fractures. Follow-up consisted of 90 fractures (90/134, 67%), at a median of five years (interquartile range (IQR) 52 to 80 months).

The median Short-Form 12 physical component score (PCS) of 62 individuals retaining their limb was 45 (IQR 36 to 53), significantly lower than the median of 51 (IQR 46 to 54) in patients who underwent delayed amputation after attempted reconstruction ($p = 0.0351$).

Regression analysis identified three variables associated with a poor F&A score: negative Bohler's angle on initial radiograph; coexisting talus and calcaneus fracture; and tibial plafond fracture in addition to a hindfoot fracture. The presence of two out of three variables was associated with a significantly lower PCS compared with amputees (medians 29, IQR 27 to 43 vs 51, IQR 46 to 54; $p < 0.0001$).

Conclusions

At five years, patients with reconstructed hindfoot fractures have inferior outcomes to those who have delayed amputation. It is possible to identify injuries which will go on to have particularly poor outcomes.

Cite this article: *Bone Joint Res* 2018;7:131–138.

Keywords: Military, Calcaneal fracture, Amputation and salvage, Hindfoot, Limb salvage, Amputation, Outcome

■ P. M. Bennett, MB ChB MRCS, Trauma & Orthopaedic Registrar,
■ T. Stevenson, MBBS MRCS, Trauma & Orthopaedic Registrar,
■ J. G. Penn-Barwell, MB ChB FRCS, Orthopaedic Trauma Surgeon, Royal Navy, Institute of Naval Medicine, Crescent Road, Alverstoke, Hampshire, PO12 2DL, UK.

■ I. D. Sargeant, MBBS FRCS OBE, Trauma & Orthopaedic Consultant, Royal Air Force, Royal Centre for Defence Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH, UK.

■ A. Mountain, MB ChB FRCS, Trauma & Orthopaedic Consultant, Royal Army Medical Corps, University Hospital of North Midlands NHS Trust, Stoke-on-Trent, ST4 6QG, UK.

Correspondence should be sent to P. M. Bennett; email: pmbennett@doctors.org.uk

doi: 10.1302/2046-3758.72.BJR-2017-0217.R2

Bone Joint Res 2018;7:131–138.

Article focus

- What are the medium-term functional outcomes of British military casualties sustaining a hindfoot fracture in combat?
- Is it possible to determine which injury features, identifiable at time of wounding, are associated with a poor functional outcome?

Key messages

- At five years, patients with reconstructed hindfoot fractures have inferior quality-of-life outcomes compared with those requiring delayed amputation following their injury.
- Three key injury features, identifiable at time of wounding, seem to be associated with particularly poor functional

outcomes: negative Böhler's angle on initial radiograph; combination of talar and calcaneal fracture; and combination of hindfoot and tibial plafond fracture.

Strengths and limitations

- This study provides surgeons with objective criteria to guide decision making when treating patients with severely injured hindfeet. The authors do not mandate that the presence of all three key injury variables should lead to amputation, but rather that the data provided should be used when counselling patients about their likely outcomes.
- Five-year follow-up is limited to 68% of all casualties sustaining a combat hindfoot fracture.
- At time of follow-up, patients were in their 20s and 30s. It is probable that their functional demands will be different later in life, and the benefits of limb salvage *versus* amputation at that time may change.

Introduction

The use of landmines and improvised explosive devices (IEDs) in areas of conflict can result in life-changing injuries not only to military personnel, but also to civilians.^{1,2} The injuries sustained by survivors of blast and gunshot wounds vary; from traumatic amputation, to complex fractures and severe soft-tissue damage.^{3,4}

The precise pattern of wounds depends on the distance of the individual from the point of detonation, whether they were on foot or in a vehicle, and whether the detonation took place in an enclosed or open space.⁵ Additionally, the use of appropriate personal protective equipment (PPE) can have a significant effect on the lower limb injuries sustained.⁶

For British and American military surgeons caring for casualties from the recent conflicts, high-energy hindfoot fractures have surpassed open tibial fractures as the greatest test in terms of the salvage and reconstruction of severe lower limb injuries.^{7,8} However, for surgeons working in certain African, Asian and Middle Eastern countries, these injuries have been a challenge for decades, and will likely continue to be long into the future.⁹

The task of achieving a painless, plantigrade, functional foot, with united fractures and healed wounds, is multifaceted. The multiple articular surfaces in the hindfoot,¹⁰ vulnerable soft-tissue envelope,¹¹ and tenuous blood supply all contribute to the reconstructive difficulties. Consequently, attention has focused on the technical aspects of limb salvage, with various orthopaedic and plastic surgical techniques examined for their use in increasing the potential for treating these injuries.¹²

Focusing on whether these injuries could be reconstructed, a function of technical feasibility, does not address whether they should be. The latter point examines whether, following a severe hindfoot injury, an

individual would achieve a greater quality of life with a reconstructed hindfoot or the alternative of transtibial amputation. This management equipoise between amputation and reconstruction depends not only on the patient's injuries, but also on the medical facilities available to treat them. Thus, an injury seen in a British soldier evacuated to the Royal Centre of Defence Medicine in Birmingham (United Kingdom) and treated by military surgeons, will be managed very differently from the same injury treated by a civilian surgeon working in an active or former conflict zone.

This study aimed to establish patients' medium-term functional recovery following severe combat hindfoot trauma. Additionally, it sought to examine the possibility of predicting, at time of injury, which injuries may progress to a poorer eventual recovery than those with amputated limbs.

Patients and Methods

This was a retrospective telephone-based follow-up study of a previously identified case series.⁷ The study was registered with, and approved by, the United Kingdom Joint Medical Command. All surviving British military casualties from the 12 years of conflict in Iraq and Afghanistan with a fracture of either the talus, calcaneus, or both, were identified.⁷ Data were gathered on patients' demographics, injury details and surgical management; patients managed with a primary amputation in the first three surgical episodes were excluded. The severity of the injury was recorded using the New Injury Severity Score (NISS).¹³ This considers the three most severe injuries sustained by the patient, regardless of body region. The total is calculated as the sum of the squares of the three most severe Abbreviated Injury Scale (AIS) injuries, to a maximum of 75.

Patients were contacted by telephone and invited to participate in the study. Those who consented were asked about their recovery, and whether they had undergone amputation or unplanned further surgery. Unplanned surgery was defined as surgery to address ongoing symptoms after definitive fixation and wound closure, i.e., revision fixation, removal of symptomatic metalwork, or fusion. All participants were assessed with the United Kingdom adapted second version of the Short-Form 12 (SF-12 V2) questionnaire,¹⁴ which ranges from 0 (lowest level of health) to 100 (highest level of health). In addition, those who had retained their limb completed the American Academy of Orthopaedic Surgeons (AAOS) foot and ankle (F&A) outcomes questionnaire (range from 0 (most disability) to 100 (least disability)).¹⁵ The AAOS F&A questionnaire was chosen as it does not require clinical assessment and can be administered over the telephone, and has been shown to correlate well with other quality-of-life outcome measures.¹⁶

Statistical analysis. Descriptive data are presented as medians with interquartile ranges (IQR). Statistical analysis

Table I. Analysis of characteristics of patients lost to follow-up

	Amputation (n = 28)	Limb salvage (n = 62)	Lost to FU (n = 44)
Age (yrs)			
Median	25	25	24
IQR	21 to 17	22 to 29	21 to 26
Mechanism of injury			
Blast (direct)	23	54	39
Blast (indirect)	2	1	2
GSW	3	5	2
Other	0	2	1
New Injury Severity Score			
Median	12	12	12
IQR	12 to 17	9 to 17	8 to 17
Open injury	13	19	18
Nerve injury	1	1	1
Arterial injury	4	4	1

FU, follow-up; IQR, interquartile range; GSW, gun shot wound

was performed with the use of SPSS v.23 software (IBM Corp., Armonk, New York). The chi-squared test was used to examine the relationship between dichotomous variables.

Injury features that would be available for the treating surgeon in their assessment of the severity of the hindfoot injury at time of presentation, were identified. A multivariate regression model was developed to examine whether these variables were subsequently associated with a lower AAOS F&A score in those patients retaining their limbs. A new model was fitted which included only those variables that had a statistically significant association, on multivariate comparison, with AAOS F&A outcome. The following seven variables were examined:

- Negative Böhler's angle on initial radiograph;
- Coexisting talar and calcaneal fracture;
- Fracture of the tibial plafond in addition to a hindfoot fracture;
- Fracture of the mid-foot in addition to a hindfoot fracture;
- Open fracture;
- Nerve injury;
- Vascular injury.

The Spearman's correlation coefficient test was used to establish the relationship between the AAOS F&A score and SF-12 score. The threshold for significance was set at a p-value < 0.05. Böhler's angle, i.e. the radiological angle between the superior tip of the calcaneal tuberosity, and the superior edge of the anterior and posterior facets, was measured on lateral radiographs taken at the time of injury.¹⁷ Nerve injury was defined as confirmed transection of a nerve identified at time of surgical exploration. Vascular injury was defined as arterial injury requiring surgical repair. The accuracy of statistical comparisons was calculated using a *post hoc* power analysis.

Table II. Indication for amputation (n = 28)

Indication	n (%)
Pain	19 (68)
Quality of life	4 (14)
Nonunion	2 (7)
Infection	2 (7)
Soft-tissue breakdown	1 (3.5)

Results

A previously reported series identified all 114 British military casualties with 134 fractured hindfeet from the conflicts in Iraq and Afghanistan.⁷ A total of 77 patients (77/114, 68%) were successfully contacted and consented to participate in this follow-up study. Patients undergoing amputation, those with retained limbs, and those lost to follow-up were similar with respect to age, mechanism of injury, and associated injuries (Table I). A total of 13 of patients followed up had bilateral injuries, providing follow-up for 90 injured limbs (90/134, 67%), with a median follow-up of 64 months (IQR 52 to 80).

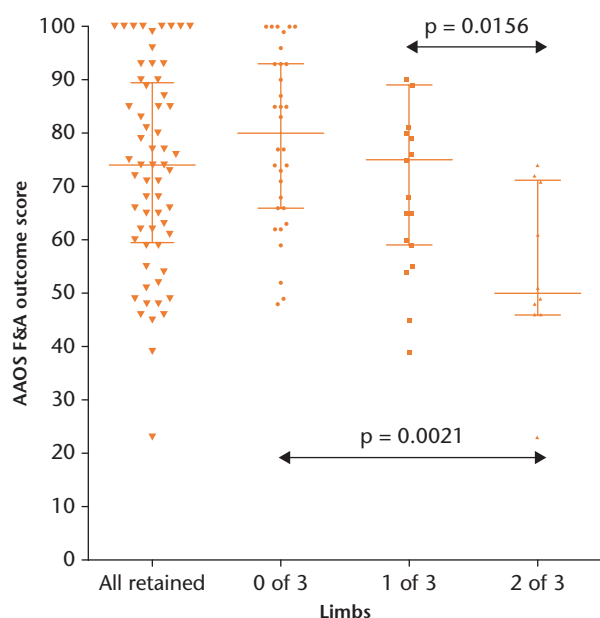
Following hindfoot reconstruction, 28 limbs (28/90, 31%) subsequently underwent amputation at a median of 14 months (IQR 11 to 21) from time of injury. A total of 19 limbs (19/28, 68%) had undergone at least one further unplanned operation after the initial hospital admission prior to eventual amputation. In 19 of the subsequent amputation cases (19/28, 67%), pain was cited as the predominant reason to elect for amputation, with further detail given in Table II.

In the 62 cases where the limb was retained, the median AAOS F&A score was 74 (IQR 61 to 88), with eight patients scoring a maximum 100 points. The multivariable regression analysis identified three key variables associated with a significantly poorer AAOS F&A score: negative Böhler's angle on initial radiograph; coexisting talar and calcaneal fracture; and fracture of the tibial plafond in addition to hindfoot fracture, with detail given in Table III. Whether the hindfoot fracture was open, the presence of a concurrent midfoot fracture, vascular or

Table III. Multivariable regression analysis demonstrating effect of injury variable on American Academy of Orthopaedic Surgeons Foot and Ankle (AAOS F&A) outcome score in individuals retaining their limb

Injury variable	Change in AAOS F&A score	p-value	95% confidence intervals	
			Lower limit	Upper limit
Negative Böhler's angle	-16 points	0.008	-27.8	-4.4
Coexisting talar and calcaneal fracture	-12 points	0.026	-24.3	-2.6
Tibial plafond fracture in addition to hindfoot fracture	-10 points	0.030	-20.1	-1.0

$F(3,57) = 6.95$ ($p < 0.0005$) R^2 0.27

**Fig. 1**

Graph showing the variation in American Academy of Orthopaedic Surgeons Foot and Ankle (AAOS F&A) score depending on presence of key variables in initial injury. Key variables: negative Böhler's angle on initial radiograph; coexisting talar and calcaneal fracture; fracture of tibial plafond in addition to hindfoot fracture.

neurological injury was not associated with subsequent AAOS F&A score.

Of the 90 limbs followed up, only one possessed all three of these key variables; this limb was amputated 20 months after the injury due to the development of osteomyelitis, despite attempts to treat this deep infection surgically. Of the 62 patients retaining their limb, the median AAOS F&A score was significantly associated with the number of variables present ($p = 0.0021$, Kruskal-Wallis). In the 19 patients whose injury exhibited one variable, the AAOS F&A score was 75 (IQR 60 to 87); this reduced to a median of 50 (IQR 47 to 59) in the ten patients whose injuries featured two variables ($p = 0.0156$, Mann-Whitney), as shown in Figure 1 and Table IV. Despite the reduction in AAOS F&A score, injuries characterized by one or more of the key variables were not associated with an increased rate of amputation ($p = 0.1717$, Fisher's exact test. Power calculation 0.96).

A comparison of AAOS F&A score and SF-12 physical component score (PCS) was performed, confirming the

positive correlation between AAOS F&A score and overall quality of life (Spearman's $r = 0.7277$, 95% CI 0.58 to 0.83) as shown in Figure 2.

The median SF-12 PCS of all 62 individuals retaining their limb was 45 (IQR 36 to 53): this was significantly lower than the median of 51 (IQR 46 to 54) in the 28 patients undergoing an amputation after initial salvage of their hindfoot injury ($p = 0.0351$, Mann-Whitney), as shown in Figure 3. This poorer outcome following salvage, compared with delayed amputation, is more pronounced if the salvaged limbs are grouped according to the presence of one or more of the three variables identified in the regression model. The cohort with two of three variables has a median SF-12 PCS of 29 (IQR 27 to 43), a score which is 22 points lower than that seen in patients who underwent amputation following initial salvage; this difference is statistically significant ($p < 0.0001$, Mann-Whitney; Table IV) and is represented graphically in Figure 4.

There was no difference in the SF-12 mental component score between those retaining their limb (median 44, IQR 39 to 47) and those undergoing amputation (median 39, IQR 30 to 47; $p = 0.133$, Mann-Whitney), though this comparison was underpowered (0.6).

Discussion

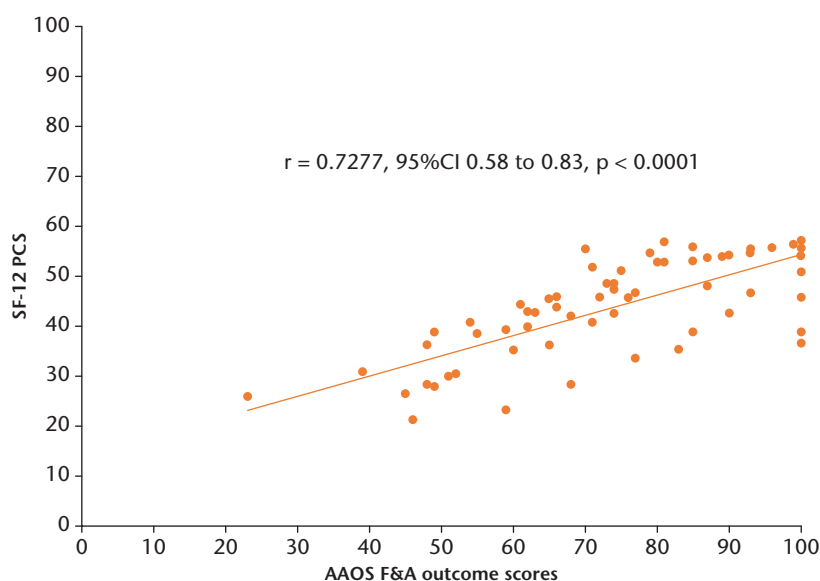
This study provides medium-term patient-reported outcomes for severe hindfoot injuries sustained in combat. Our results show that patients undergoing a transtibial amputation following initial salvage of their hindfoot injury have significantly higher physical outcome scores than those retaining their limb. Furthermore, our results demonstrate that three key variables, identifiable at time of injury, are associated with profoundly poorer functional outcome: negative Böhler's angle on initial radiograph; coexisting talar and calcaneal fracture; and fracture of the tibial plafond in addition to hindfoot fracture.

The three key variables identified in this study which are associated with poor functional outcome arguably equate to injury severity and complexity. It is therefore unsurprising that injuries with these variables have worse functional outcomes than those without. However, it is noteworthy that injuries with soft-tissue disruption and neurovascular compromise, typically regarded as important features of injury severity, are not associated with a poorer outcome.

Table IV. Comparison of outcome measures for primary amputees, delayed amputees and salvaged lower limb injuries

Cohort	Follow-up mths (IQR)	Median AAOS F&A score (IQR)	Median SF-12 PCS (IQR)
Initial salvage			
Retained limb	64 (52 to 80)	-	-
Overall	-	74 (61 to 88)	45 (36 to 53)
1/3 key variables* (n = 19)	-	75 (60 to 87)	-
2/3 key variables* (n = 10)	-	50 (47 to 59)	29 (27 to 43)
Delayed amputation	64 (52 to 80)	-	51 (46 to 54)

*Key variables: negative Böhler's angle on initial radiograph; coexisting talar and calcaneal fracture; fracture of tibial plafond in addition to hindfoot fracture
IQR, interquartile range; AAOS F&A, American Academy of Orthopaedic Surgeons Foot and Ankle; SF-12, Short-Form 12; PCS, physical component score

**Fig. 2**

Graph showing the correlation between American Academy of Orthopaedic Surgeons Foot and Ankle (AAOS F&A) score and Short-Form 12 (SF-12) physical component score (PCS). (Spearman's correlation coefficient test).

The variables used in the multivariable regression analysis were chosen as they were identifiable at the time of injury. Furthermore, they have either previously been shown to be associated with eventual outcome,¹⁸ or were thought by the treating surgeons to be associated with poor functional outcome during routine follow-up. Associations with other injury variables, such as deep infection and wound management, have previously been discussed in the literature,^{19,20} but are not identifiable at time of injury, and therefore have limited use when considering whether limb salvage should be attempted. Though wound size and location have also been examined for association with outcome,²¹ these measures are subjective, and affected by the amount of debridement required during the initial procedures.

The question of whether severe hindfoot fractures sustained in combat could be salvaged successfully has previously been reported by the authors.⁷ The results of this study contribute to addressing whether these injuries should be salvaged where the focus is on subsequent quality of life, rather than technical feasibility of surgical reconstruction.²²

Previous studies have attempted to evaluate the impact of hindfoot injuries sustained in combat. Sheehan et al²³ described a cohort of 122 patients, using return to duty (RTD) rates as a surrogate marker of disability. Although RTD rates have been used in several studies relating to military patients,^{24,25} their use is limited by not determining the functional demands of that patient's duty, and any workplace adaptations made for them. Helgeson et al²⁶ described factors associated with subsequent requirement for amputation after attempted salvage of combat-related hindfoot injury, though their follow-up was limited to subjective outcome measures.

Many surgeons would regard amputation later than the first or second procedure as a failure of salvage. However, in this study, amputation was regarded as a failure of salvage only if it was required after three procedures due to established patient management protocols. The first surgical procedure would be damage control aimed purely at saving life and, if possible, limbs, and would be limited to a washout, debridement and stabilization. Immediately upon arrival back in the United Kingdom, a second look in theatre would allow for assessment of

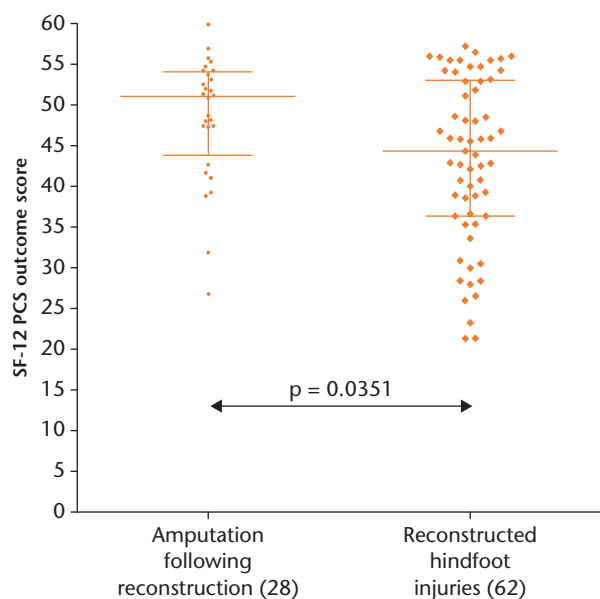


Fig. 3

Graph showing the comparison between Short-Form 12 physical component score (SF-12 PCS) outcome scores for patients with reconstructed hindfoot injuries, and those requiring amputation following attempted reconstruction.

viability and operative planning. The third procedure was therefore often the definitive one, where amputation or fracture fixation for salvage would be undertaken. The period between injury and definitive operative procedure is typically one week. Using similar methodology, some authors have previously reported on outcomes in casualties sustaining a primary lower limb amputation during the same conflicts.²⁷ In that group, the median SF-36 PCS of transtibial amputees was 47 (IQR 42 to 55) at a mean follow-up of 40 months (range 25 to 75).

Multiple scoring systems have been developed to guide surgical decision making over amputation *versus* salvage,²⁸⁻³² though none has had their clinical use validated.³³ These scoring systems were developed in civilian populations, and though attempts have been made to translate them to a military setting,³⁴ their use remains unproven.³⁵

It is important to address some of the non-medical confounders in assessing recovery following severe limb trauma in military patients. There is a hierarchy of combat injuries, both about financial compensation, and about the attitude of fellow service personnel and wider society. This was illustrated starkly in studies of United States veterans of the Vietnam war that revealed that bilateral transfemoral amputees reported a higher quality of life than their comrades with unilateral limb loss.^{36,37} It is suspected that these financial and social factors might make injured service personnel more inclined towards amputation rather than salvage. Additionally, it is possible that the increased compensation soldiers receive for losing their limb may 'artificially' elevate scores in quality-of-life measures.

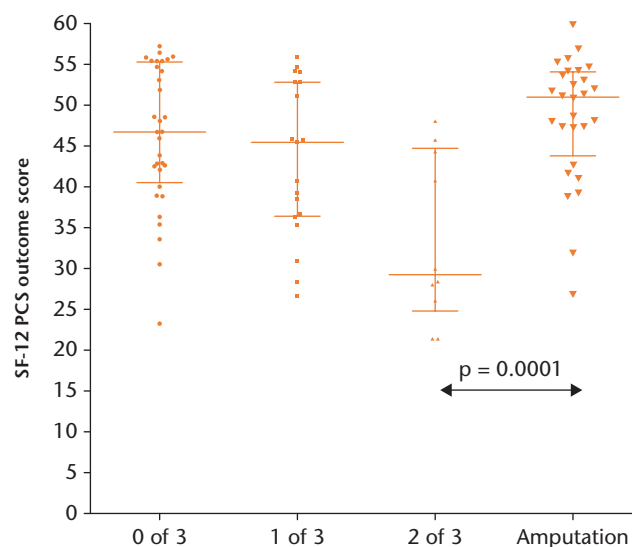


Fig. 4

Graph showing the variation in Short-Form 12 physical component (SF-12 PCS) outcome scores for those with reconstructed hindfoot injuries depending on presence of key variables in initial injury, compared with those patients requiring delayed amputation following attempted reconstruction. Key variables: negative Böhler's angle on initial radiograph; coexisting talar and calcaneal fracture; fracture of tibial plafond in addition to hindfoot fracture.

The difficulties of giving patients with severe hindfoot injuries a realistic expectation of their likely outcome, and how this might change over their lifetime, is understood.³⁸ All British service personnel injured overseas are repatriated to a single unit in the United Kingdom. This centralized care has the benefit of a single team of surgeons managing these complex injuries, which allows for the accumulation of experience and the evolution of surgical techniques.

These findings indicate that it might be possible in some hindfoot injuries to identify which fractures would have an inferior functional outcome with salvage, rather than amputation. However, the authors strongly advise against the adoption of an 'algorithmic' decision-making process. These findings provide the clinician with a greater evidential basis for advising and counselling patients as to their likely outcomes with each of the two alternative treatment strategies.³⁹

The heterogeneous nature of these fractures, combined with ipsilateral or more proximal injuries and personal preference, mandates an individualized approach to each patient. The relatively low R^2 coefficient achieved by the multivariable regression model clearly shows that these variables are only describing about a third of the factors influencing recovery. When considering surgical options, subjective assessment of both the injury and patient by an experienced reconstructive surgical team and rehabilitation specialists will continue to be fundamental in advising patients of the treatment options and likely recovery.⁴⁰

The findings of this study appear to be consistent with the recently reported results of the Outcomes After Severe

Distal Tibia, Ankle, and/or Foot Trauma: Comparison of Limb Salvage Versus Transtibial Amputation trial (OUTLET), a prospective, observational multicentre study of North American civilian patients.^{41,42} There were 410 patients in the limb salvage group and 87 in the transtibial amputation group who completed the Short Musculoskeletal Function Assessment (SMFA) at 18 months. Patients with reconstructed severe distal tibia, and/or mid- or hindfoot injuries reported significantly poorer scores across most SMFA domains, particularly mobility, compared with those treated with transtibial amputation.

There are weaknesses in this study. Follow-up was only available for 68% of the patients with hindfoot fractures from Iraq and Afghanistan, although those lost to follow-up appeared to be similar to those included, in terms of demographics and injury severity. The loss of follow-up of a third of the patients relates to the mobile and migrant nature of a young military population, especially those who have sustained a life-changing injury. Furthermore, the R^2 coefficient of the multivariable regression analysis suggests an incomplete understanding of the causes behind patients' poor functional outcome. The use of the AAOS F&A scoring system appears to have a ceiling, as clearly shown in Figure 2, with respect to measuring recovery in a small number of servicemen, and this might limit its use in this population of patients.

Nearly all of these patients were reporting on their quality of life when still in their 20s and 30s. It is possible that the superior outcome scores in the amputee cohort might be reversed in favour of the salvage group when these patients are in their 60s and 70s. The size of the cohort of patients precluded any evaluation of associations between outcome scores and age or time to follow-up.

It is inappropriate to apply these findings directly to civilian trauma patients due to the differences in mechanisms of injury and patient demographic. Though civilian hindfoot fractures are considered a high-energy injury, the massive soft-tissue damage, gross contamination and comminution seen in military patients, who are almost exclusively young men injured because of blast and gunshot injuries, distinguishes the two cohorts. However, the 'could' versus 'should' management dilemma of these injuries is relevant to all surgeons treating victims of blast and gunshot wounds, and this equipoise will vary depending on the environment in which the patient is being treated.

Despite these weaknesses, this work provides medium-term patient-reported outcome measures for patients sustaining severe hindfoot fractures in combat, and identifies key variables at time of injury to assist surgical decision making and patient counselling. The clear finding that there is a group of patients with hindfoot injuries that are technically salvageable, but who might have a superior outcome with a transtibial amputation, should

be recognized by all surgeons managing these challenging injuries.

References

1. Arul G, Reynolds J, DiRusso S, et al. Paediatric admissions to the British military hospital at Camp Bastion, Afghanistan. *Ann R Coll Surg Engl* 2012;94:52-57.
2. Jacobs N, Taylor DM, Parker PJ. Changes in surgical workload at the JF Med Gp Role 3 Hospital, Camp Bastion, Afghanistan, November 2008-November 2010. *Injury* 2012;43:1037-1040.
3. Bilukha OO, Laurence H, Danee L, Subedi KP, Becknell K. Injuries and deaths due to victim-activated improvised explosive devices, landmines and other explosive remnants of war in Nepal. *Inj Prev* 2011;17:326-331.
4. Korver A. Injuries of the lower limbs caused by antipersonnel mines: the experience of the International Committee of the Red Cross. *Injury* 1996;27:477-479.
5. Kang DG, Lehman RA, Carragee EJ. Wartime spine injuries: understanding the improvised explosive device and biophysics of blast trauma. *Spine J* 2012;12: 849-857.
6. Hayda R, Harris RM, Bass CD. Blast injury research: modeling injury effects of landmines, bullets, and bombs. *Clin Orthop Relat Res* 2004;422:97-108.
7. Bennett PM, Stevenson T, Sargeant ID, Mountain A, Penn-Barwell JG. Salvage of Combat Hindfoot Fractures in 2003-2014 UK Military. *Foot Ankle Int* 2017;38:745-751.
8. Penn-Barwell JG, Bennett PM, Fries CA, et al. Severe open tibial fractures in combat trauma: management and preliminary outcomes. *Bone Joint J* 2013;95-B: 101-105.
9. Cluster Munition Coalition. International campaign to ban landmines. *Landmine Monitor* 2017 <http://www.the-monitor.org/en-gb/our-research/landmine-monitor.aspx> (date last accessed 10 January 2018).
10. Sarrafian SK. Biomechanics of the subtalar joint complex. *Clin Orthop Relat Res* 1993;290:17-26.
11. Levin LS, Nunley JA. The management of soft-tissue problems associated with calcaneal fractures. *Clin Orthop Relat Res* 1993;290:151-156.
12. Poon H, Le Cocq H, Mountain AJ, Sargeant ID. Dermal Fenestration With Negative Pressure Wound Therapy: A Technique for Managing Soft Tissue Injuries Associated With High-Energy Complex Foot Fractures. *J Foot Ankle Surg* 2016;55:161-165.
13. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *J Trauma* 1997;43:922-925.
14. Jenkinson C, Layte R. Development and testing of the UK SF-12. *J Health Serv Res Policy* 1997;2:14-18.
15. Tran T, Thordarson D. Functional outcome of multiply injured patients with associated foot injury. *Foot Ankle Int* 2002;23:340-343.
16. Johanson NA, Liang MH, Daltroy L, Rudicel S, Richmond J. American Academy of Orthopaedic Surgeons lower limb outcomes assessment instruments. *J Bone Joint Surg [Am]* 2004;86-A:902-909.
17. Böhler L. Diagnosis, pathology, and treatment of fractures of the os calcis. *J Bone Joint Surg [Am]* 1931;13-A:75-89.
18. Loucks C, Buckley R. Bohler's angle: correlation with outcome in displaced intra-articular calcaneal fractures. *J Orthop Trauma* 1999;13:554-558.
19. Benirschke SK, Kramer PA. Wound healing complications in closed and open calcaneal fractures. *J Orthop Trauma* 2004;18:1-6.
20. Berry G, Stevens D, Kreder H, et al. Open fractures of the calcaneus: a review of treatment and outcome. *J Orthop Trauma* 2004;18:202-206.
21. Dickens CPJF, Kilcoyne CKG, Kluk CMW, et al. Risk factors for infection and amputation following open, combat-related calcaneal fractures. *J Bone Joint Surg [Am]* 2013;95-A:e24.
22. Arthur CHC, Mountain AJC. The unhappy triad of ankle injury. *J Bone Joint Surg [Br]* 2012;94-B(Suppl XXXII):1.
23. Sheean AJ, Krueger CA, Hsu JR. Return to duty and disability after combat-related hindfoot injury. *J Orthop Trauma* 2014;28:e258-e262.
24. Stinner DJ, Burns TC, Kirk KL, Ficke JR. Return to duty rate of amputee soldiers in the current conflicts in Afghanistan and Iraq. *J Trauma* 2010;68:1476-1479.
25. Cohen SP, Brown C, Kurihara C, et al. Diagnoses and factors associated with medical evacuation and return to duty for service members participating in Operation Iraqi Freedom or Operation Enduring Freedom: a prospective cohort study. *Lancet* 2010;375:301-309.
26. Helgeson MD, Potter BK, Burns TC, Hayda RA, Gajewski DA. Risk factors for and results of late or delayed amputation following combat-related extremity injuries. *Orthopedics* 2010;33:669.

27. **Bennett PM, Sargeant ID, Midwinter MJ, Penn-Barwell JG.** Unilateral lower limb loss following combat injury: medium-term outcomes in British military amputees. *Bone Joint J* 2013;95-B:224-229.
28. **Hellet DL, Howey T, Sanders R, Johansen K.** Limb salvage versus amputation. Preliminary results of the Mangled Extremity Severity Score. *Clin Orthop Relat Res* 1990;256:80-86.
29. **Howe HR Jr, Poole GV Jr, Hansen KJ, et al.** Salvage of lower extremities following combined orthopedic and vascular trauma. A predictive salvage index. *Am Surg* 1987;53:205-208.
30. **McNamara MG, Heckman JD, Corley FG.** Severe Open Fractures of the Lower Extremity: A Retrospective Evaluation of the Mangled Extremity Severity Score (MESS). *J Orthop Trauma* 1994;8:81-87.
31. **Russell WL, Sailors DM, Whittle TB, Fisher DF Jr, Burns RP.** Limb salvage versus traumatic amputation. A decision based on a seven-part predictive index. *Ann Surg* 1991;213:473-480.
32. **Tscherne H, Oestern H.** A new classification of soft-tissue damage in open and closed fractures (author's transl). *Unfallheilkunde* 1982;85:111-115. (Article in German)
33. **Bosse MJ, MacKenzie EJ, Kellam JF, et al.** A prospective evaluation of the clinical utility of the lower-extremity injury-severity scores. *J Bone Joint Surg [Am]* 2001;83-A:3-14.
34. **Kjorstad R, Starnes BW, Arrington E, et al.** Application of the Mangled Extremity Severity Score in a combat setting. *Mil Med* 2007;172:777-781.
35. **Brown KV, Ramasamy A, McLeod J, Stapley S, Clasper JC.** Predicting the need for early amputation in ballistic mangled extremity injuries. *J Trauma* 2009;66: S93-S98.
36. **Dougherty PJ.** Long-term follow-up of unilateral transfemoral amputees from the Vietnam war. *J Trauma* 2003;54:718-723.
37. **Dougherty PJ.** Long-Term Follow-up Study of Bilateral Above-the-Knee Amputees from the Vietnam War. *J Bone Joint Surg [Am]* 1999;81-A:1384-1390.
38. **Rossiter ND, Higgins TF, Pallister I.** (ii) The mangled extremity: limb salvage versus amputation. *Orthop Trauma* 2014;28:137-140.
39. **Wordsworth M, Lawton G, Nathwani D, et al.** Improving the care of patients with severe open fractures of the tibia. *Bone Joint J* 2016;98-B:420-424.
40. **Glen J, Constanti M, Brohi K, Guideline Development Group.** Assessment and initial management of major trauma: summary of NICE guidance. *BMJ* 2016;353:i3051.
41. **Bosse MJ, Teague D, Reider L, et al.** Outcomes After Severe Distal Tibia, Ankle, and/or Foot Trauma: Comparison of Limb Salvage Versus Transtibial Amputation (OUTLET). Orthopaedic Trauma Association Annual Meeting. Vancouver, Canada, 2017.
42. **Bosse MJ, Teague D, Reider L, et al.** METRC. Outcomes After Severe Distal Tibia, Ankle, and/or Foot Trauma: Comparison of Limb Salvage Versus Transtibial Amputation (OUTLET). *J Orthop Trauma* 2017;31(Suppl 1):S48-S55.

Funding Statement

- Fees for publication were borne by the primary and senior authors' unit affiliation, the Institute of Naval Medicine

Author Contributions

- P. M. Bennett: Study design and conception, data collection, statistical analysis, manuscript preparation.
- T. Stevenson: Data collection.
- I. D. Sargeant: Surgical oversight, data collection.
- A. Mountain: Surgical oversight, data collection.
- J. G. Penn-Barwell: Study design and conception, data analysis, manuscript preparation.

Conflict of Interest Statement

- None declared

© 2018 Bennett et al. This is an open-access article distributed under the terms of the Creative Commons Attributions licence (CC-BY-NC), which permits unrestricted use, distribution, and reproduction in any medium, but not for commercial gain, provided the original author and source are credited.

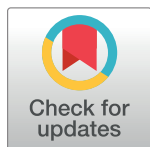
RESEARCH ARTICLE

Preclinical therapies to prevent or treat fracture non-union: A systematic review

Philippa M. Bennett^{1*}, Sarah K. Stewart², Janine Dretzke³, Danai Bem³, Jowan G. Penn-Barwell¹

1 Institute of Naval Medicine, Crescent Road, Alverstoke, Hampshire, United Kingdom, **2** Royal Centre for Defence Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham, United Kingdom, **3** Institute of Applied Health Research, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom

* pippa_bennett@icloud.com



Abstract

Background

Non-union affects up to 10% of fractures and is associated with substantial morbidity. There is currently no single effective therapy for the treatment or prevention of non-union. Potential treatments are currently selected for clinical trials based on results from limited animal studies, with no attempt to compare results between therapies to determine which have the greatest potential to treat non-union.

Aim

The aim of this systematic review was to define the range of therapies under investigation at the preclinical stage for the prevention or treatment of fracture non-union. Additionally, through meta-analysis, it aimed to identify the most promising therapies for progression to clinical investigation.

Methods

MEDLINE and Embase were searched from 1st January 2004 to 10th April 2017 for controlled trials evaluating an intervention to prevent or treat fracture non-union. Data regarding the model used, study intervention and outcome measures were extracted, and risk of bias assessed.

Results

Of 5,171 records identified, 197 papers describing 204 therapies were included. Of these, the majority were only evaluated once (179/204, 88%), with chitosan tested most commonly (6/204, 3%). Substantial variation existed in model design, length of survival and duration of treatment, with results poorly reported. These factors, as well as a lack of consistently used objective outcome measures, precluded meta-analysis.

OPEN ACCESS

Citation: Bennett PM, Stewart SK, Dretzke J, Bem D, Penn-Barwell JG (2018) Preclinical therapies to prevent or treat fracture non-union: A systematic review. PLoS ONE 13(8): e0201077. <https://doi.org/10.1371/journal.pone.0201077>

Editor: Wisit Cheungpasitporn, University of Mississippi Medical Center, UNITED STATES

Received: June 6, 2018

Accepted: July 8, 2018

Published: August 1, 2018

Copyright: © 2018 Bennett et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Costs for publication were met by a grant from UNIFY, Engineering and Physical Sciences Research Council, EP/N027221/1.

Competing interests: The authors have declared that no competing interests exist.

Conclusion

This review highlights the variability and poor methodological reporting of current non-union research. The authors call for a consensus on the standardisation of animal models investigating non-union, and suggest journals apply stringent criteria when considering animal work for publication.

Introduction

Fracture non-union can be defined as occurring when the normal healing processes of bone cease to the extent that solid healing cannot occur without further intervention[1]. The condition is estimated to affect 5–10% of fractures[2, 3], with wide variation depending on anatomical location[4]. The negative effect on quality of life associated with non-union has been demonstrated as being greater than that of diabetes mellitus, stroke and acquired immunodeficiency syndrome[5], with substantial financial consequences[6].

The failure of a fracture to unite is multifactorial and the result of both predisposing and contributing factors[1, 7]. There is no consensus or accepted guidelines for the treatment of non-union, but most current management strategies involve hospital admission and revision surgery, frequently using bone graft or synthetic substitutes, with varied and unpredictable results. In order to either primarily prevent non-union, increase the likelihood of success of revision surgery, or potentially offer an alternative to surgery, researchers continue to evaluate novel therapies in this field.

Preclinical studies are defined as those using animals to determine if a treatment is likely to be effective, before progression to testing in humans [8].

It is currently not clear on what basis researchers select potential therapies for translation into clinical studies. It is likely that positive results from a single, or a small number, of animal studies are used to justify progression to clinical trial. However, it is problematic to rely on the positive effects of a therapy in a single animal study to justify direct translation to clinical testing due to the likely existence of bias and methodological weakness. There is no evidence that researchers in this field have compared different preclinical studies in an attempt to determine which therapies are the most promising and therefore should be prioritised for translation into clinical studies.

Systematic reviews summarise the literature for a defined research question; when combined with a meta-analysis of results they are considered to represent the highest level in the hierarchy of evidence[9]. Despite this, meta-analyses are reliant upon the quality of data in the original studies included, and can risk propagating any errors included in the original research. The methodology for systematic reviews of preclinical research is still evolving, but it is recognised that the technique has the potential to clarify the existing evidence base and potentially increase the precision of effect estimates through meta-analysis[10, 11]. To date there has not been a systematic review or meta-analysis of preclinical studies aiming to prevent or treat fracture non-union.

The aim of this systematic review was firstly to establish the range of therapies under investigation at the preclinical stage for the prevention or treatment of fracture non-union. Secondly, by conducting a meta-analysis of results of methodologically similar studies, it aimed to systematically and objectively identify the most promising therapies for progression to clinical investigation.

Materials and methods

Search strategy and inclusion criteria

Full methodological details can be found in the previously published protocol[12]. The protocol was registered with Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES)[13]. A summary of the methods is reported below. Reporting of the full systematic review was in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[14], (S1 Table).

MEDLINE and Embase were searched via Ovid from 1st January 2004 to 10th April 2017 (see S2 Table for full search strategy). The citation lists of included studies were searched for additional studies. In a deviation from the methodology published in the study protocol, due to the large volume of studies retrieved from the primary searches, no further additional sources were searched.

Two reviewers (PMB/SKS) independently screened titles and abstracts. Where eligibility for inclusion could not be determined from the abstract the full manuscript was obtained and reviewed for clarification. Any disagreements were resolved through discussion with a third reviewer (JPB). Controlled trials evaluating an intervention to prevent or treat non-union and measuring bone formation were eligible for inclusion; the focus of this review was to examine preclinical therapies with clinical potential and so treatments which had already been evaluated in a clinical study were excluded. Full inclusion and exclusion criteria were listed in the previously published protocol and are summarised in Table 1. Relevant preclinical studies evaluating therapies that had subsequently progressed to clinical trial were excluded, unless the therapy was combined with a novel therapy.

After duplicates were removed, 5,171 records were identified in the literature search as shown in the PRISMA flow diagram (Fig 1). After inclusion/exclusion criteria were applied 197 studies were included in the systematic review. The commonest single reason for study exclusion (1,073 studies, 21%) was that the article described a therapy that had already progressed to clinical trial.

Table 1. Summary of study inclusion and exclusion criteria.

Inclusion Criteria	
Types of studies	Controlled trials
	Unpublished and published works
Types of participants	Mammalian model testing an intervention to treat or prevent fracture non-union
	Induced co-morbidities
Intervention	Interventions aim to:
	• Prevent non-union
	• Treat non-union
	• Promote or accelerate healing of a bony defect
	• Treat or ameliorate delayed union
	Administered after formation of a bony defect
	Established interventions in a novel vehicle
Comparator	Control group described receiving:
	• No treatment
	• Current standard of care
	• Alternative treatment
Outcome measures	Quantifiable measure of bone formation through radiological and/or histological means
Exclusion Criteria	
Types of studies	Review articles
Types of participants	Clinical trials
Intervention	Any intervention that has subsequently progressed to clinical trial

<https://doi.org/10.1371/journal.pone.0201077.t001>

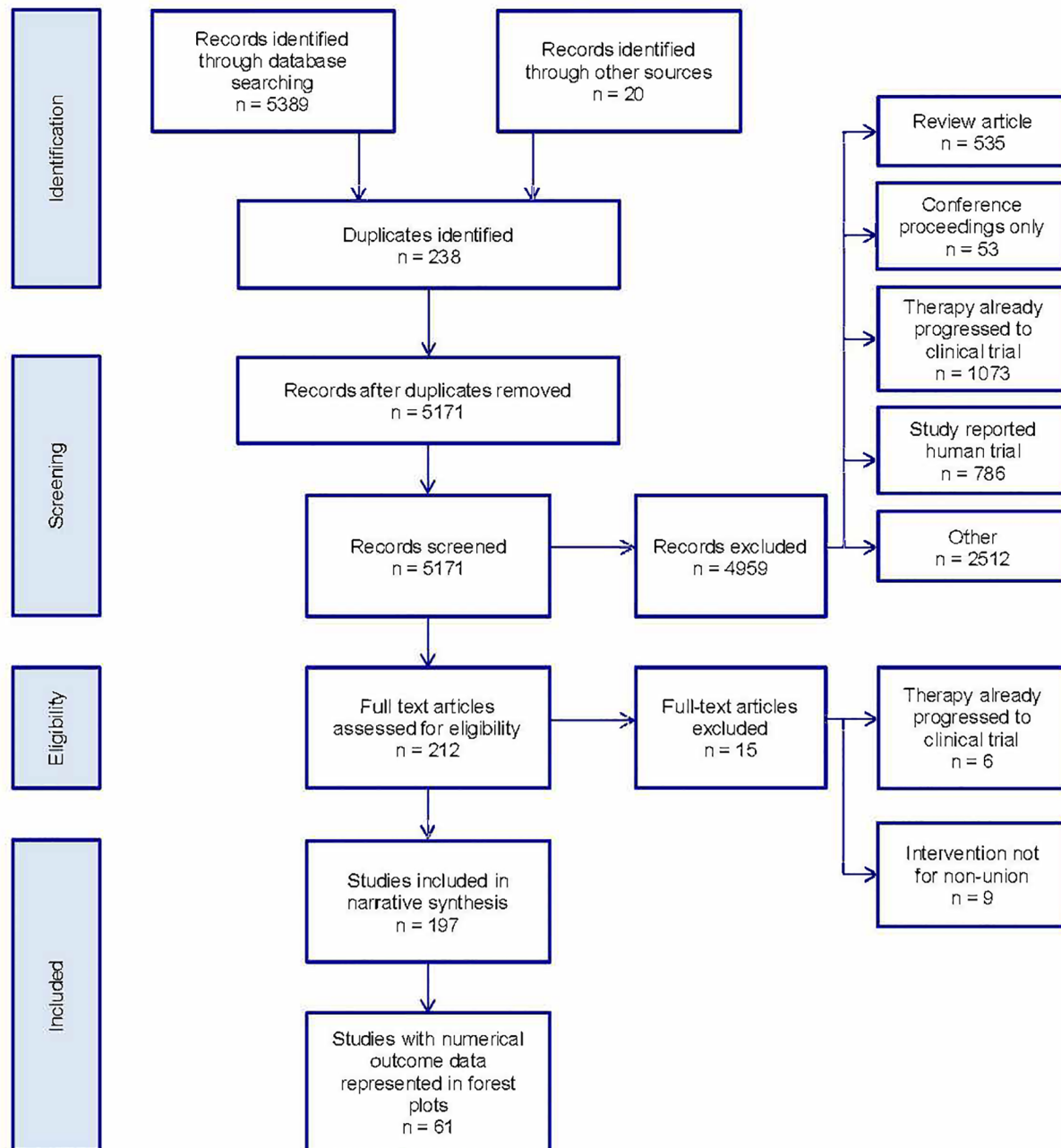


Fig 1. PRISMA flow diagram for study inclusion/exclusion. Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) flow diagram detailing numbers of studies excluded and reasons at each stage of the review process.

<https://doi.org/10.1371/journal.pone.0201077.g001>

Data extraction and risk of bias assessment

Data relating to the model, defect location and method of creation, length of survival, number of animals included, outcome measures (radiological or histological) were extracted from manuscripts.

Where incomplete data was provided in the manuscript authors were contacted for clarification: of the 64 authors contacted, only 9 replied with the required information (14%).

Numerical data extraction from papers presenting results in graphical format only was performed using ImageJ v.2.0 software (National Institute of Health, Bethesda, MD) using a standardised method[15, 16].

The Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) risk of bias tool was used to assess risk of bias across all studies[17]. The SYRCLE tool assesses ten domains across six types of bias: selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding), detection bias (random outcome assessment, blinding), attrition bias (incomplete outcome data), reporting bias (selecting outcome reporting) and other sources of bias. Risk of bias assessment was performed by one author (PMB or SKS). Each domain was given a rating of high risk, low risk or unclear where information was incomplete or not reported. These ratings were based on the signalling questions designed to assist judgement, as detailed in the SYRCLE tool[17].

Analysis

Where studies reported sufficient data (numbers in intervention and control group, mean and standard deviation), results for the most consistently reported measures (bone formation (%), bone volume (mm³) or bone density (mg/cm³)) were represented in forest plots for illustrative purposes. Results for the remaining studies were tabulated. Where several time-points were reported, only the longest follow-up was considered.

Therapies were grouped into the following nine categories:

- Animal derivatives
- Plant extracts
- Minerals/elements/chemicals
- Pharmaceuticals
- Cells/tissues
- Vibration/motion
- Light/lasers
- Gases
- Human proteins/hormones

If a therapy related to more than one category, it was included in both it pertained to (e.g. mesenchymal stem cells with insulin-like growth factor-1 was recorded in both the 'cells/tissues' and 'human proteins/hormones' categories.) Combination therapies using both an established therapy already in clinical trial with a novel preclinical therapy were again recorded in both categories to which they pertained.

Results

The spectrum of potential treatments

The 197 included studies evaluated a total of 204 different interventions (Table 2). The objective of approximately half of all studies was to promote or accelerate healing of a bony defect (103/197, 52%) or treat non-union (93/103, 47%), with further information available in S3 Table. The majority of therapies (179/204 (88%)) were only evaluated once, while five interventions (chitosan [18–23], adipose stromal cells [24–27], erythropoietin [28–31], vascular endothelial growth factor [32–35] and SDF-1 [36–38]) were investigated by multiple studies

Table 2. Number of evaluations under investigation by category*.

Group	Number of evaluations included in tables	Number of evaluations included in forest plots	Total
Animal derivatives	27	5	32
Plant extracts	23	13	36
Minerals / elements / chemicals	25	7	32
Pharmaceuticals	16	13	29
Cells / tissues	32	18	50
Vibration / motion	2	5	7
Light / lasers	3	0	3
Gases	3	5	8
Human proteins / hormones	59	41	100
Total	190	107	297

*Combination therapies are duplicated in **all** groups they pertain to, e.g. mesenchymal stem cells + vascular endothelial growth factor will be counted in “cells / tissues” and “human proteins / hormones”.

Single therapies tested in multiple concentrations are counted more than once, e.g. Ngueguim 2012 evaluates two plant based therapies: both therapies are evaluated at three different concentrations, thereby contributing 6 evaluations.

A total of 197 studies were included, investigating a total of 204 distinct therapies.

Total number of studies included in tables = 136, total number of studies included in forest plots = 61.

<https://doi.org/10.1371/journal.pone.0201077.t002>

(Table 3). Chitosan as a single therapy was evaluated by six studies: four of these found significantly greater bone formation in the intervention group compared to control [18, 20–22], with further detail in Table 3.

Risk of bias

Details necessary to assess risk of bias were vastly underreported, particularly with regard to random housing, random outcome assessment (randomisation), sequence generation,

Table 3. Most frequently evaluated therapies across all studies (n = 197).

Therapy	Number of studies evaluating therapy	Direction of effect
Chitosan	6	Four studies [18, 20–22] favoured intervention over control. One study [19] favoured control over intervention. One study [23] showed no difference between intervention and control.
Adipose stromal cells	4	Two studies [25, 27] favoured intervention over control. Two studies [24, 26] showed no difference between intervention and control.
Erythropoietin	4	Four studies [28–31] showed no difference between intervention and control
Vascular endothelial growth factor	4	Two studies [32, 35] favoured intervention over control. Two studies [33, 34] showed no difference between intervention and control.
SDF-1	3	Two studies [36, 38] favoured intervention over control. One study [37] showed no difference between intervention and control.
Therapies tested twice	40	
Therapies tested once	179	

<https://doi.org/10.1371/journal.pone.0201077.t003>

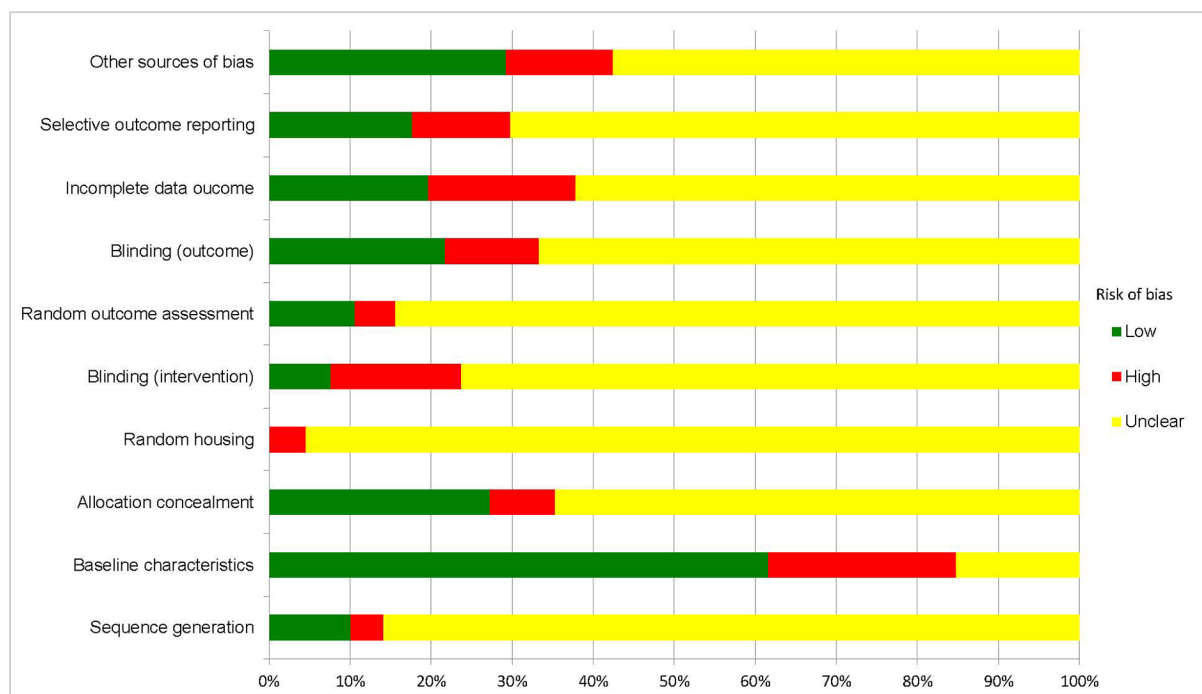


Fig 2. Risk of bias analysis. Bias assessed as per the Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) tool for all 197 studies included.

<https://doi.org/10.1371/journal.pone.0201077.g002>

blinding of outcome assessment and selective outcome reporting (Fig 2). Between 4 and 23% of studies were judged to be at high risk of bias for a given criterion. No study reported details for all ten domains of the SYRCLE tool.

The most consistently reported outcome measure was percentage bone formation in the category of human proteins and hormones (Fig 3 [25, 28, 32, 36, 39–58]). Study findings across all categories for bone formation, bone volume and bone density are shown in Fig 4, [23, 47, 51, 53, 54, 57, 59–77], Fig 5 [29, 37, 38, 78–86] and Fig 6 [87–91]). Table 4 ([92–105]) shows the findings for the pharmaceutical therapies that could not be represented in forest plots, with findings for the remaining categories available as supporting information (S4, S5, S6, S7, S8, S9, S10 and S11 Tables).

In total 53 human protein and hormone therapy evaluations (30 in forest plots, 23 in tables, 53/100, 53%) reported statistically significant improvements in bone healing compared to the control groups. Statistically significant improvements for the other categories were 50% animal derivatives (16/32), 53% plant extracts (19/36), 55% minerals/elements/chemicals (18/33), 38% pharmaceuticals (11/29), 54% cells/tissues (26/48), 30% vibration/motion (3/10), 100% light/lasers (3/3) and 75% gases (6/8). In total, 135 separate therapy evaluations (135/204, 66%) showed a significantly greater effect on healing of fracture non-union when compared to the control. Only a minority of interventions (9/204, 4%) resulted in significantly less effect on bone union than the comparator arm.

Meta-analysis

Substantial heterogeneity across studies in terms of type and site of defect, method of defect creation, species, length of follow-up and method of outcome reporting precluded meta-analysis.

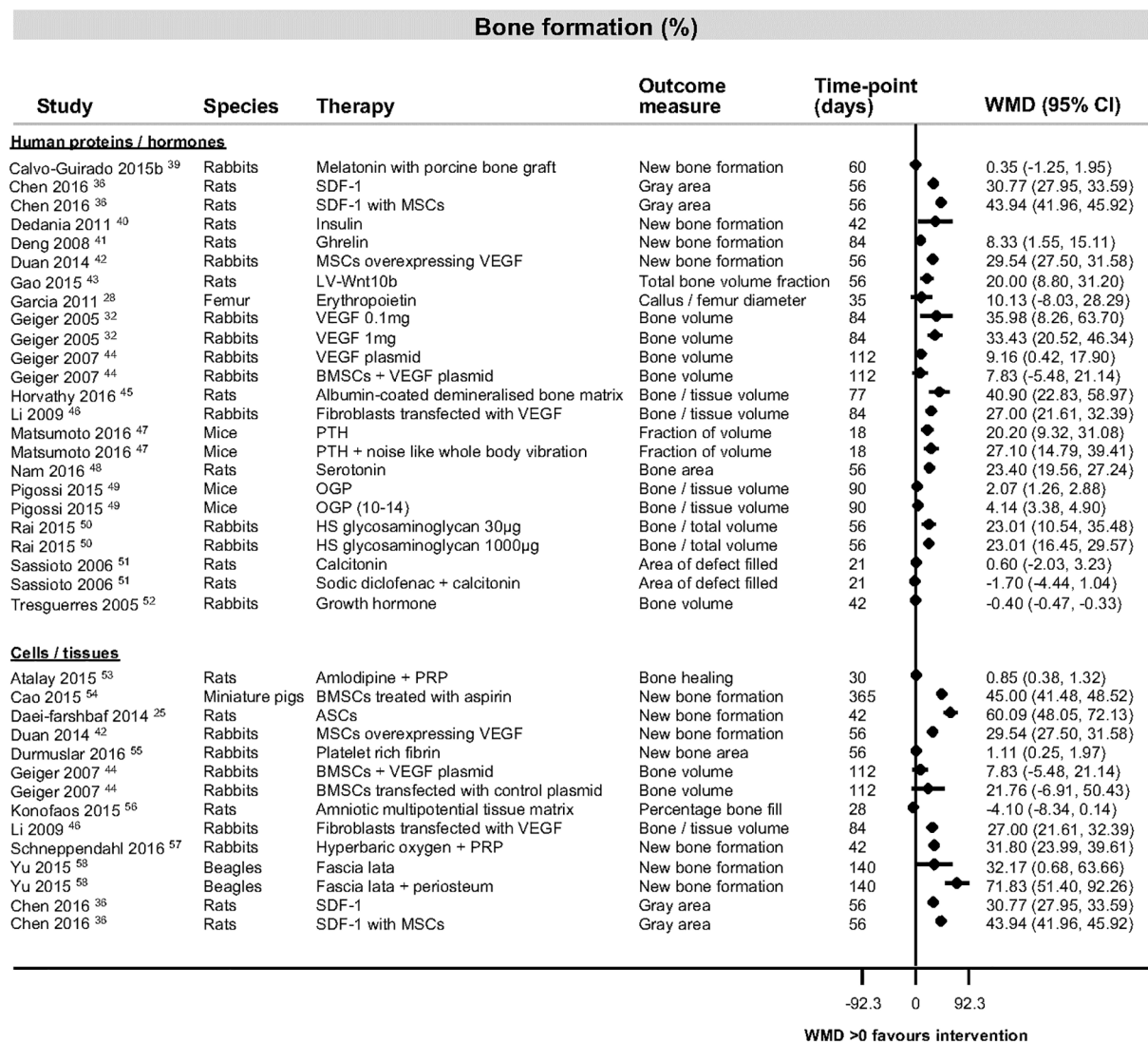


Fig 3. Bone formation data for studies looking at interventions of human proteins and hormones or cells and tissues. Forest plot illustrating mean difference in percentage of bone formation as measured by different histological or radiological measures. Abbreviations: ASCs, adipose tissue stem cells; BMSCs, bone marrow stromal cells; CI, confidence interval; HS, heparan sulphate; LV-Wnt10b, lentivirus vector encoding Wnt10b gene; MSCs, mesenchymal stem cells; OGP, osteogenic growth peptide; PRP, platelet rich plasma; PTH, parathyroid hormone; SDF-1, stromal cell derived factor 1; VEGF, vascular endothelial growth factor; WMD, weighted mean difference.

<https://doi.org/10.1371/journal.pone.0201077.g003>

Rats were the most common animal model, used in 105 studies (105/197, 53%), with the calvarium being the commonest site of bony defect (71/197, 36%). Pigs, dogs, goats, rabbits and mice were also used. Further detail on animal and defect location is given in Table 5. It was not possible to determine the total number of animals used in 28 studies (28/197, 14%) with further detail in S2 Table. Studies used both radiological and histological outcome measures, with follow-up times ranging from 1–30 weeks (Fig 7).

Regarding the defect, the majority of studies (75/197, 38%) did not report how the defect was created. A bur was used in 51 studies (51/197, 26%), with other methods including drills (14%), saws (12%), three-point bending (5%), drop weights or pendulums (3%), and being cut with scissors (3%). The defect was explicitly stated as being critical in 75 studies (75/197, 38%)

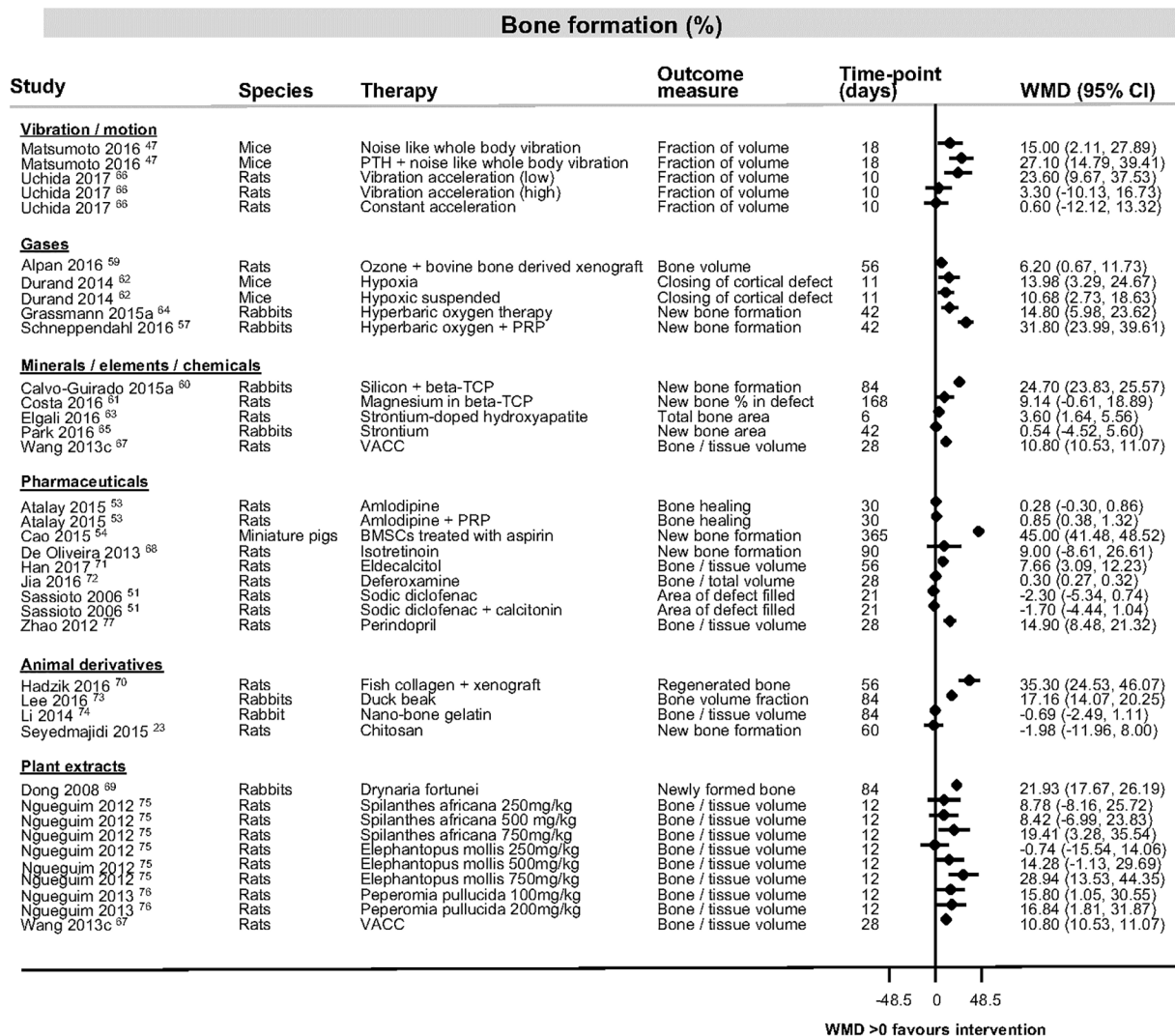


Fig 4. Bone formation data for studies looking at interventions of vibration and motion, gases, minerals, elements and chemicals, pharmaceuticals, animal derivatives or plant extracts. Forest plot illustrating mean difference in percentage of bone formation as measured by different histological or radiological measures. Abbreviations: BMSCs, bone marrow stromal cells; CI, confidence interval; PRP, platelet rich plasma; PTH, parathyroid hormone; VACC, vanadium absorbed by Coprinus comatus; WMD, weighted mean difference.

<https://doi.org/10.1371/journal.pone.0201077.g004>

and non-critical in 2 (2/197, 1%), with the remainder of studies (155/197, 79%) not providing this detail. Ten studies (6%) cauterised or stripped the periosteum surrounding the osteotomy.

Only one third of studies (61/197, 31%) included sufficient data to permit illustration in forest plots (without quantitative pooling), due to insufficient reporting of outcome data, or use of less commonly used outcome metrics.

Discussion

Fracture non-union is a common complication of a common condition [1–3]. This systematic review highlights not only the range of research activity in this field but the poor quality of contemporary animal research investigating this condition. Meta-analysis was not possible due to the diverse and non-standardised nature of the preclinical research, range of outcome measures and poor reporting of results. Despite there being a large amount of data– 204

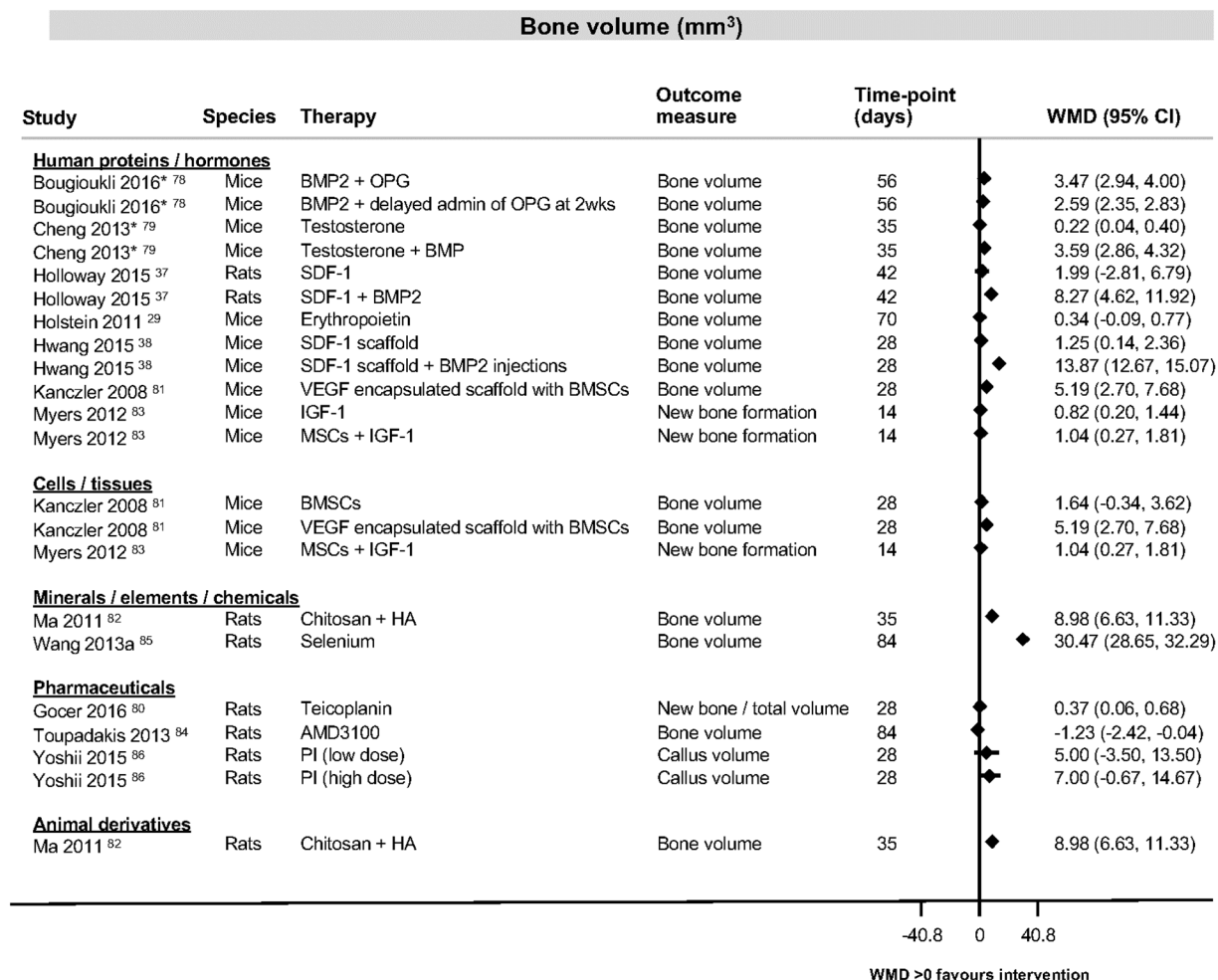


Fig 5. Bone volume data for studies looking at interventions of human proteins and hormones, cells and tissues, minerals, elements and chemicals, pharmaceuticals or animal derivatives. Forest plot illustrating mean difference in cubic millimetre (mm³) of bone volume as measured by different histological or radiological measures. *Since none of the control groups healed, the increase in bone volume was set as 0 and the standard deviation as 0.0000001 in order to be able to illustrate those results in a forest plot using STATA. Abbreviations: BMP2, bone morphogenetic protein 2; BMSCs, bone marrow stromal cells; CI, confidence interval; HA, hyaluronic acid; IGF-1, insulin growth factor-1; MSCs, mesenchymal stem cells; OPG, osteoprotegerin; PI, proteasome inhibitor; SDF-1, stromal cell derived factor 1; VEGF, vascular endothelial growth factor; wks, weeks; WMD, weighted mean difference.

<https://doi.org/10.1371/journal.pone.0201077.g005>

evaluations across 197 studies—it has not been possible to make a valid comparison between any two studies nor draw firm conclusions regarding relative efficacies from different interventions and therefore identify those therapies that should be prioritised in translational research.

When developing preclinical models of fracture non-union various factors need to be considered. Fundamentally these include the species of animal to be used and the anatomical location of the fracture. Additionally, the type of fracture (transverse or segmental), whether it is subsequently stabilised or not and whether or not the periosteum is stripped are all variables that will affect the union rates of the fracture model. Finally, the delivery method of the therapy under investigation, including the use of scaffolds and carriers, must also be considered. The greater the number of differences that exist between model designs, the less reliably any differences in union rates can be attributed to the therapy under investigation alone, as model variations will act as confounders.

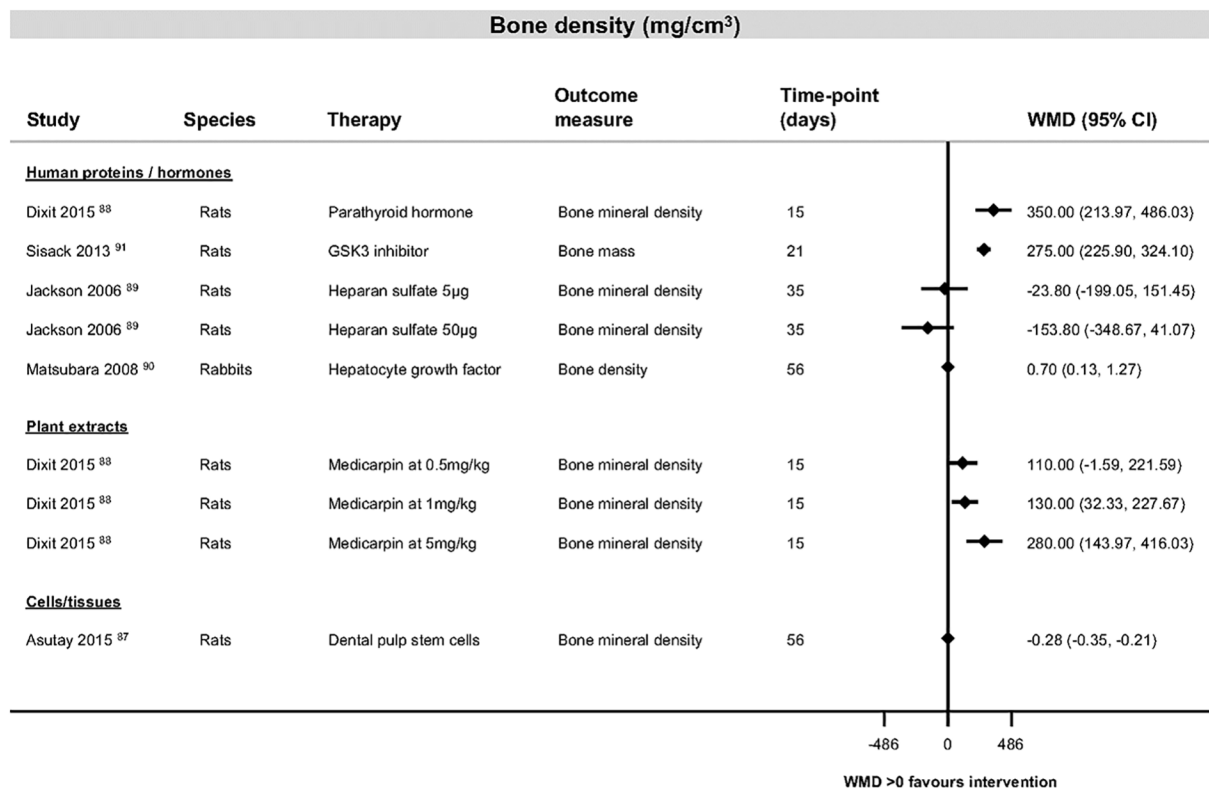


Fig 6. Bone density data for studies looking at interventions of human proteins and hormones, cells and tissues or plant extracts. Forest plot illustrating mean difference in milligrams per cubic centimetre (mg/cm³) of bone density as measured by different histological or radiological measures. Abbreviations: CI, confidence interval; GSK3, glycogen synthase kinase 3; WMD, weighted mean difference.

<https://doi.org/10.1371/journal.pone.0201077.g006>

In clinical practice the progression of a fracture to established non-union is multi-factorial, with different types of non-union existing. The majority of primary research contained within this systematic review failed to consider this variability during model development: though the stated aim was to test a therapy designed to prevent or treat non-union, very few used proven models of non-union. The poor fidelity to clinical situations further limits the utility of the pre-clinical findings.

This systematic review used a methodologically rigorous approach to identifying, selecting and appraising primary studies. There were however some deviations from the previously published protocol; the authors chose to use the MEDLINE version of PUBMED to allow easier duplication of the search strategy on OVID. The decision to limit the systematic review to only these two primary databases was made due to the large volume of eligible studies included. The authors judged it unlikely that the inclusion of a small number of additional studies identified through other sources would significantly alter any conclusions, particularly given the variable and methodologically poor reporting of studies identified in the main databases. Additionally, the large number of studies meant that the risk of bias assessment was performed by one reviewer only for each study.

The studies included in this systematic review were limited by inadequate reporting of methodological details and results. Applying the risk of bias tool developed by SYRCLE showed that many risk of bias criteria were not reported and the rating of 'unclear' risk of bias was most common. This in turn hampers interpretation of results. It is however in line with the findings of previous studies which found poor reporting of randomisation

Table 4. Defect repair data for studies evaluating therapies based on pharmaceuticals (16 therapies, 14 studies).

Study	Therapy	Species	Maximum length of survival (days)	Outcome	Overall effect
Alic 2016 [92]	Cilostazol	Rats	21	No difference between groups at end of 21 days	=
Baht 2017 [93]	Nefopam	Mice	21	Treatment with Nefopam resulted in fracture calluses that contained higher proportions of bone and lower proportions of fibrous tissue	→
Bernick 2014 [94]	Lithium	Rats	28	Fracture healing was maximised with low dose, later onset and longer treatment duration of lithium, resulting in significantly greater yield torque in the therapeutic group	↑
Cai 2015 [95]	Lithium	Rabbits	84	New bone area for lithium containing mesoporous bioglass markedly higher than that for lithium containing bioglass at 56 and 84 days	→
Cakmak 2015 [96]	Pentoxifylline	Rats	56	No bone growth in control or systemic pentoxifylline only groups	=
Cakmak 2015 [96]	Pentoxifylline + iliac crest autograft	Rats	56	Radiological bone union was observed in the iliac crest autograft and systemic pentoxifylline group compared to no new bone growth in the control group	→
Del Rosario 2015 [97]	Simvastatin	Rats	56	No significant difference between groups	=
Donneys 2013 [98]	Deferoxamine	Rats	40	Greater union rate in treatment group than in irradiated group, but both less than control group	↓
Fan 2017 [99]	Phenamil	Rats	86	Incomplete mandibular restoration was observed in the defect treated with phenamil alone	?
Fan 2017 [99]	Phenamil + BMP	Rats	86	Addition of BMP to phenamil synergistically augmented bone healing, resulting in almost complete bone healing	→
Ishack 2017 [100]	Dipyridamole	Mice	56	Significant increase in percentage of bone regenerated in dipyridamole group compared to control group	↑
Kutan 2016 [101]	Doxycycline	Rats	28	Osteogenesis in the test group was significantly higher than that of the control group	↑
Limirio 2016 [102]	Doxycycline + alendronate	Rats	15	Statistically greater bone density in therapeutic group compared to control group at 15 days	↑
Wada 2013 [103]	Salicylic acid	Rats	84	Significantly higher new bone in defect in therapeutic group compared to control group	↑
Werkman 2006 [104]	Risedronate	Rats	28	No significant difference between therapeutic and control groups	=
Wixted 2009 [105]	Zileuton	Mice	28	Net increase in callus size relative to control	→

↑ indicates statistically significant effect on bone formation in trial therapy compared to control

→ indicates greater bone formation in trial therapy compared to control, but the effect did not reach statistical significance

= indicates no difference in bone formation rates between the therapeutic or control groups

↓ indicates less effect on bone formation in trial therapy compared to control

? indicates results are unclear, and no effect size could be determined

<https://doi.org/10.1371/journal.pone.0201077.t004>

Table 5. Model of non-union by species and anatomical location.

	Calvarium	Femur	Humerus	Mandible	Radius	Rib	Tibia	Ulna	Zygomatic arch	Total
Pigs	2									2
Dogs					1		1			2
Goats							1			1
Rabbits	17	8	1	2	14		10	4	1	57
Rats	42	40		6	1	1	14	1		105
Mice	10	12					8			30
Total	71	60	1	8	16	1	34	5	1	197

<https://doi.org/10.1371/journal.pone.0201077.t005>

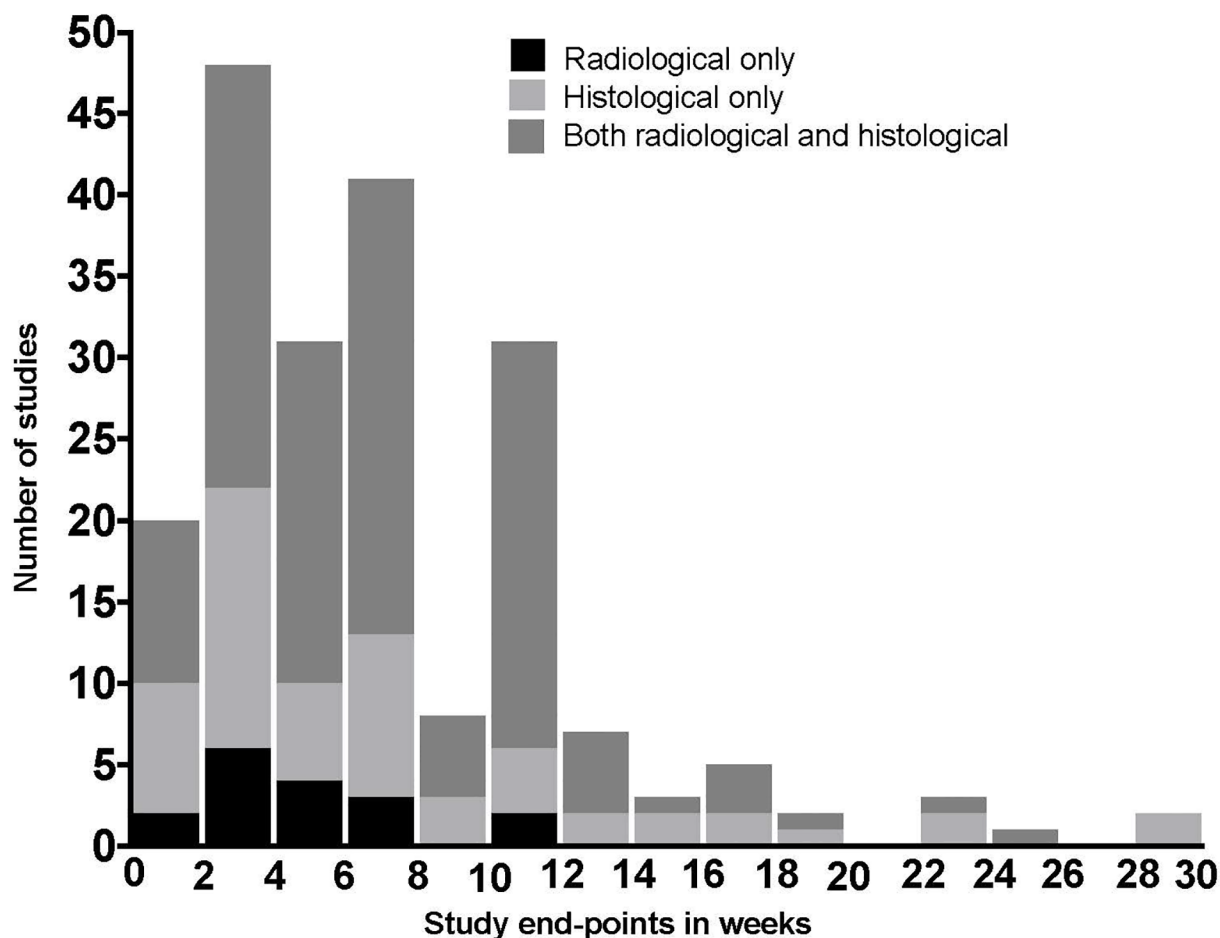


Fig 7. Bar graph demonstrating varied study methodology. Illustration of study-end point in weeks and outcome measure used by all 197 studies.

<https://doi.org/10.1371/journal.pone.0201077.g007>

procedures and blinding of assessors in animal studies[106], despite multiple resources for study design and reporting available to researchers[107–109]. Some omissions were extremely basic, for example 11% of studies had to be excluded from the forest plots for not stating whether their results were reported as mean with standard deviation, or standard error of the mean, with authors failing to provide clarification when contacted. The use of \pm in methodological reporting without further explanation has previously been identified as a persistent problem[110, 111].

To address the problems identified by this review, the authors recommend that the orthopaedic trauma community attempt to reach a consensus on preferred animal models of bone healing similar to the standardisation of fracture classification with the OTA/AO/Muller system[112]. Once a consensus on the standardisation of species, defect and outcome measure is achieved, funding could be restricted to researchers using agreed models and study methodology[113], and journals should similarly restrict publication to studies that would allow direct comparison and insist on reporting results in detail. However, even if this were achieved, the translatability of animal research into effective clinical trials remains controversial [114–116], with even highly cited animal studies failing to translate into successful interventions in clinical trials[117].

This systematic review describes the diverse range of treatments currently under investigation at the preclinical stage for the prevention or treatment of fracture non-union. These therapies can be divided into nine broad treatment categories. Approximately 90% of interventions were only evaluated by a single study, and only five were evaluated three or more times. Reliance on a single study is problematic given the methodological limitations of the research and when considered in the context of publication bias.

Publication bias is an established problem of clinical trials, and its prevalence in animal studies is increasingly recognised[115, 118]. Failing to publish non-significant results of pre-clinical research limits the ability of researchers to interpret the efficacy of a therapy in the context of the wider literature. It is also unethical: subjecting animals to experiments without publishing the results effectively wastes those animals. The majority of studies included in this review (66%) reported significantly greater rates of bone healing in the therapeutic group compared to the control group. While formal assessment of publication bias was not possible it is reasonable to speculate that a bias against publication of negative or non-significant results persists.

The variability across studies meant that no two studies from the 197 included in this review were judged to be sufficiently similar across clinical and methodological parameters to allow pooling of results in a meta-analysis. Only 31% of studies presented their results in sufficient detail to be illustrated graphically in a forest plot. Not only does this preclude a rapid visual comparison of results from different studies, but it is also indicative of a lack of detail in reporting scientific findings.

Heterogeneity is expected in systematic reviews of preclinical research. Indeed, it could be argued that the aim of a systematic review in this field is to explore and demonstrate the breadth of the evidence, the variability between studies and the consistency of any findings. The generation of a precise pooled effect estimate through meta-analysis even where this is deemed feasible may be of limited value given translatability issues. Yet in this review it was mostly not possible to comment on the consistency of benefit of a particular intervention, as they were mostly only explored in one or two studies.

This systematic review has defined the considerable range of therapies currently being investigated at the preclinical phase for the treatment and prevention of fracture non-union. Though some studies report statistically significant results for some therapies, high levels of clinical and methodological heterogeneity and poor methodological quality and reporting severely hamper the ability to prioritise therapies for translation into clinical trials. If the orthopaedic trauma community were to collectively agree on a standardised animal model for investigating this question, and standards for reporting of all results regardless of findings were mandated, improved clinical treatments for fracture non-union will be developed more efficiently.

Supporting information

S1 Table. PRISMA checklist. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist for reporting of study methodology, results and discussion. (DOC)

S2 Table. Systematic review search strategies for MEDLINE and Embase. (DOCX)

S3 Table. Additional study detail. (DOCX)

S4 Table. Defect repair data for studies evaluating therapies based on animal derivatives (27 therapies, 18 studies).

(DOCX)

S5 Table. Defect repair data for studies evaluating therapies based on plant extracts (23 therapies, 23 studies).

(DOCX)

S6 Table. Defect repair data for studies evaluating therapies based on minerals, elements or chemicals (25 therapies, 21 studies).

(DOCX)

S7 Table. Defect repair data for studies evaluating therapies based on cells and tissues (32 therapies, 27 studies).

(DOCX)

S8 Table. Defect repair data for studies evaluating therapies based on vibration or motion (2 therapies, 2 studies).

(DOCX)

S9 Table. Defect repair data for studies evaluating therapies based on lights or lasers (3 therapies, 2 studies).

(DOCX)

S10 Table. Defect repair data for studies evaluating therapies based on gases (3 therapies, 3 studies).

(DOCX)

S11 Table. Defect repair data for studies evaluating therapies based on human proteins or hormones (59 therapies, 42 studies).

(DOCX)

Acknowledgments

We thank Professor Sarah Stapley FRCS for her contribution to the protocol phase of this study.

Author Contributions

Conceptualization: Philippa M. Bennett, Janine Dretzke, Danai Bem, Jowan G. Penn-Barwell.

Formal analysis: Philippa M. Bennett, Janine Dretzke, Danai Bem.

Investigation: Philippa M. Bennett, Sarah K. Stewart, Jowan G. Penn-Barwell.

Methodology: Philippa M. Bennett, Janine Dretzke, Danai Bem, Jowan G. Penn-Barwell.

Project administration: Philippa M. Bennett.

Resources: Philippa M. Bennett.

Supervision: Jowan G. Penn-Barwell.

Writing – original draft: Philippa M. Bennett.

Writing – review & editing: Philippa M. Bennett, Janine Dretzke, Danai Bem, Jowan G. Penn-Barwell.

References

1. Brinker M. Nonunions: Evaluation and Treatment. In: Browner BD, Jupiter JB, Levine AM, Trafton PG, editors. *Skeletal Trauma Basic Science, Management, and Reconstruction*. One. Sixth ed. Philadelphia: Saunders; 2003.
2. Calori G, Mazza E, Colombo M, Ripamonti C, Tagliabue L. Treatment of long bone non-unions with polytherapy: indications and clinical results. *Injury*. 2011; 42(6):587–90. <https://doi.org/10.1016/j.injury.2011.03.046> PMID: 21524745
3. Zura R, Xiong Z, Einhorn T, Watson JT, Ostrum RF, Prayson MJ, et al. Epidemiology of fracture non-union in 18 human bones. *JAMA surgery*. 2016; 151(11):e162775–e. <https://doi.org/10.1001/jamasurg.2016.2775> PMID: 27603155
4. Tzioupis C, Giannoudis PV. Prevalence of long-bone non-unions. *Injury*. 2007; 38:S3–S9.
5. Schottel PC, O'Connor DP, Brinker MR. Time trade-off as a measure of health-related quality of life: long bone nonunions have a devastating impact. *JBJS*. 2015; 97(17):1406–10.
6. Hak DJ, Fitzpatrick D, Bishop JA, Marsh JL, Tilp S, Schnettler R, et al. Delayed union and nonunions: epidemiology, clinical issues, and financial aspects. *Injury*. 2014; 45:S3–S7.
7. Calori G, Albisetti W, Agus A, Iori S, Tagliabue L. Risk factors contributing to fracture non-unions. *Injury*. 2007; 38:S11–S8.
8. National Cancer Institute. NCI Dictionary of Cancer Terms <https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=44517> [cited 2017]. <https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=44517>.
9. Cook DJ, Mulrow CD, Haynes RB. Systematic reviews: synthesis of best evidence for clinical decisions. *Annals of internal medicine*. 1997; 126(5):376–80. PMID: 9054282
10. Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I, Group RATS. Where is the evidence that animal research benefits humans? *BMJ: British Medical Journal*. 2004; 328(7438):514. <https://doi.org/10.1136/bmj.328.7438.514> PMID: 14988196
11. Sandercock P, Roberts I. Systematic reviews of animal experiments. *The Lancet*. 2002; 360(9333):586.
12. Stewart SK, Bennett PM, Stapley SA, Dretzke J, Bem D, Penn-Barwell JG. Pre-clinical evaluation of therapies to prevent or treat bone non-union: a systematic review protocol. *Systematic reviews*. 2015; 4(1):161.
13. Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies (CAMARADES). <http://www.dcn.ed.ac.uk/camarades/default.htm> <http://www.dcn.ed.ac.uk/camarades/default.htm> [13 August 2017].
14. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS medicine*. 2009; 6(7):e1000097. <https://doi.org/10.1371/journal.pmed.1000097> PMID: 19621072
15. Oliveira IR, Santos-Jesus R, Po ALW, Poolsup N. Extracting numerical data from published reports of pharmacokinetics investigations: method description and validation. *Fundamental & clinical pharmacology*. 2003; 17(4):471–2.
16. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular reproduction and development*. 2015; 82(7–8):518–29. <https://doi.org/10.1002/mrd.22489> PMID: 26153368
17. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYR-CLE's risk of bias tool for animal studies. *BMC medical research methodology*. 2014; 14(1):43.
18. Azevedo AS, Sa MJ, Fook MV, Neto PI, Sousa OB, Azevedo SS, et al. Use of chitosan and beta-tricalcium phosphate, alone and in combination, for bone healing in rabbits. *Journal of Materials Science-Materials in Medicine*. 2014; 25(2):481–6. <https://doi.org/10.1007/s10856-013-5091-2> PMID: 24243224.
19. Canter HI, Vargel I, Korkusuz P, Oner F, Gungorduk DB, Cil B, et al. Effect of use of slow release of bone morphogenetic protein-2 and transforming growth factor-Beta-2 in a chitosan gel matrix on cranial bone graft survival in experimental cranial critical size defect model. *Annals of Plastic Surgery*. 2010; 64(3):342–50. <https://doi.org/10.1097/SAP.0b013e3181a73045> PMID: 20179488.
20. Cui X, Zhao D, Zhang B, Gao Y. Osteogenesis mechanism of chitosan-coated calcium sulfate pellets on the restoration of segmental bone defects. *Journal of Craniofacial Surgery*. 2009; 20(5):1445–50. <https://doi.org/10.1097/SCS.0b013e3181af1529> PMID: 19816276.
21. Ezoddini-Ardakani F, Navabazam A, Fatehi F, Danesh-Ardekani M, Khadem S, Rouhi G. Histologic evaluation of chitosan as an accelerator of bone regeneration in microdrilled rat tibias. *Dental Research Journal*. 2012; 9(6):694–9. PMID: 23559943.

22. Park SS, Kim SG, Lim SC, Ong JL. Osteogenic activity of the mixture of chitosan and particulate dentin. *Journal of Biomedical Materials Research Part A*. 2008; 87(3):618–23. <https://doi.org/10.1002/jbm.a.31812> PMID: 18186071.
23. Seyedmajidi M, Rabiee S, Haghanifar S, Seyedmajidi S, Jorsaraei SGA, Alaghehmand H, et al. Histopathological, histomorphometrical, and radiographical evaluation of injectable glass-ceramic-chitosan nanocomposite in bone reconstruction of rat. *International Journal of Biomaterials*. 2015, 2015 Article Number: 719574 Date of Publication: 2015. 2015.
24. Cheung WK, Working DM, Galuppo LD, Leach JK. Osteogenic comparison of expanded and uncultured adipose stromal cells. *Cytotherapy*. 2010; 12(4):554–62. <https://doi.org/10.3109/14653241003709694> PMID: 20370353.
25. Daei-farshbaf N, Ardeshirylajimi A, Seyedjafari E, Piryaee A, Fadaei Fathabady F, Hedayati M, et al. Bioceramic-collagen scaffolds loaded with human adipose-tissue derived stem cells for bone tissue engineering. *Molecular Biology Reports*. 41 (2) (pp 741–749), 2014 Date of Publication: February 2014. 2014. <https://doi.org/10.1007/s11033-013-2913-8> PMID: 24363224.
26. Heo SC, Shin WC, Lee MJ, Kim BR, Jang IH, Choi EJ, et al. Periostin accelerates bone healing mediated by human mesenchymal stem cell-embedded hydroxyapatite/tricalcium phosphate scaffold. *PLoS ONE*. 10 (3) (no pagination), 2015 Article Number: e0116698 Date of Publication: 16 Mar 2015.
27. Lin Y, Wang T, Wu L, Jing W, Chen X, Li Z, et al. Ectopic and in situ bone formation of adipose tissue-derived stromal cells in biphasic calcium phosphate nanocomposite. *Journal of Biomedical Materials Research Part A*. 2007; 81(4):900–10. <https://doi.org/10.1002/jbm.a.31149> PMID: 17236222.
28. Garcia P, Speidel V, Scheuer C, Laschke MW, Holstein JH, Histing T, et al. Low dose erythropoietin stimulates bone healing in mice. *Journal of Orthopaedic Research*. 29 (2) (pp 165–172), 2011 Date of Publication: February 2011. 2011. <https://doi.org/10.1002/jor.21219> PMID: 20740668.
29. Holstein JH, Orth M, Scheuer C, Tami A, Becker SC, Garcia P, et al. Erythropoietin stimulates bone formation, cell proliferation, and angiogenesis in a femoral segmental defect model in mice. *Bone*. 2011; 49(5):1037–45. <https://doi.org/10.1016/j.bone.2011.08.004> PMID: 21851867.
30. Omlor GW, Kleinschmidt K, Gantz S, Speicher A, Guehring T, Richter W. Increased bone formation in a rabbit long-bone defect model after single local and single systemic application of erythropoietin. *Acta Orthopaedica*. 87 (4) (pp 425–431), 2016 Date of Publication: 03 Jul 2016. <https://doi.org/10.1080/17453674.2016.1198200> PMID: 27348783.
31. Wan L, Zhang F, He Q, Tsang WP, Lu L, Li Q, et al. EPO promotes bone repair through enhanced cartilaginous callus formation and angiogenesis.[Erratum appears in PLoS One. 2014;9(10):e111830]. *PLoS ONE [Electronic Resource]*. 2014; 9(7):e102010. <https://doi.org/10.1371/journal.pone.0102010> PMID: 25003898.
32. Geiger F, Bertram H, Berger I, Lorenz H, Wall O, Eckhardt C, et al. Vascular endothelial growth factor gene-activated matrix (VEGF 165-GAM) enhances osteogenesis and angiogenesis in large segmental bone defects. *Journal of Bone and Mineral Research*. 20 (11) (pp 2028–2035), 2005 Date of Publication: November 2005. 2005. <https://doi.org/10.1359/JBMR.050701> PMID: 16234976.
33. Ogilvie CM, Lu C, Marcucio R, Lee M, Thompson Z, Hu D, et al. Vascular endothelial growth factor improves bone repair in a murine nonunion model. *Iowa Orthopaedic Journal*. 2012; 32:90–4. PMID: 23576927.
34. Patel ZS, Young S, Tabata Y, Jansen JA, Wong ME, Mikos AG. Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model. *Bone*. 2008; 43(5):931–40. <https://doi.org/10.1016/j.bone.2008.06.019> PMID: 18675385.
35. Cz Xu, Wg Yang, Xf He, Lt Zhou, Xk Han, Xf Xu. Vascular endothelial growth factor and nano-hydroxyapatite/collagen composite in the repair of femoral defect in rats. *Journal of Clinical Rehabilitative Tissue Engineering Research*. 15 (38) (pp 7118–7122), 2011 Date of Publication: 2011. 2011.
36. Chen G, Fang T, Qi Y, Yin X, Di T, Feng G, et al. Combined use of mesenchymal stromal cell sheet transplantation and local injection of SDF-1 for bone repair in a rat nonunion model. *Cell Transplantation*. 25 (10) (pp 1801–1817), 2016 Date of Publication: 2016. <https://doi.org/10.3727/096368916X690980> PMID: 26883892.
37. Holloway JL, Ma H, Rai R, Hankenson KD, Burdick JA. Synergistic Effects of SDF-1alpha and BMP-2 Delivery from Proteolytically Degradable Hyaluronic Acid Hydrogels for Bone Repair. *Macromolecular Bioscience*. 15 (9) (pp 1218–1223), 2015 Date of Publication: 01 Sep 2015. <https://doi.org/10.1002/mabi.201500178> PMID: 26059079.
38. Hwang HD, Lee JT, Koh JT, Jung HM, Lee HJ, Kwon TG. Sequential Treatment with SDF-1 and BMP-2 Potentiates Bone Formation in Calvarial Defects. *Tissue engineering Part A*. 21(13–14):2125–35, 2015 Jul. <https://doi.org/10.1089/ten.TEA.2014.0571> PMID: 25919507.
39. Calvo-Guirado JL, Gomez-Moreno G, Mate-Sanchez JE, Lopez-Mari L, Delgado-Ruiz R, Romanos GE. New bone formation in bone defects after melatonin and porcine bone grafts: experimental study

- in rabbits. *Clinical Oral Implants Research* 26(4):399–406, 2015 Apr. <https://doi.org/10.1111/clr.12364> PMID: 24602080.
40. Dedania J, Borzio R, Paglia D, Breitbart EA, Mitchell A, Vaidya S, et al. Role of local insulin augmentation upon allograft incorporation in a rat femoral defect model. *Journal of Orthopaedic Research*. 2011; 29(1):92–9. <https://doi.org/10.1002/jor.21205> PMID: 20661933.
41. Deng F, Ling J, Ma J, Liu C, Zhang W. Stimulation of intramembranous bone repair in rats by ghrelin. *Experimental Physiology* 93 (7) (pp 872–879), 2008 Date of Publication: July 2008. 2008. <https://doi.org/10.1113/expphysiol.2007.041962> PMID: 18562476.
42. Duan C, Liu J, Yuan Z, Meng G, Yang X, Jia S, et al. Adenovirus-mediated transfer of VEGF into marrow stromal cells combined with PLGA/TCP scaffold increases vascularization and promotes bone repair in vivo. *Archives of Medical Science* 10 (1) (pp 174–181), 2014 Date of Publication: February 2014. 2014. <https://doi.org/10.5114/aoms.2012.30950> PMID: 24701231.
43. Gao F, Zhang C, Chai Y, Li X. Lentivirus-mediated Wnt10b overexpression enhances fracture healing in a rat atrophic non-union model. *Biotechnology letters* 37 (3) (pp 733–739), 2015 Date of Publication: 01 Mar 2015. <https://doi.org/10.1007/s10529-014-1703-2> PMID: 25348747.
44. Geiger F, Lorenz H, Xu W, Szalay K, Kasten P, Claes L, et al. VEGF producing bone marrow stromal cells (BMSC) enhance vascularization and resorption of a natural coral bone substitute. *Bone*. 2007; 41(4):516–22. <https://doi.org/10.1016/j.bone.2007.06.018> PMID: 17693148.
45. Horvathy DB, Vacz G, Szabo T, Szigyarto IC, Toro I, Vamos B, et al. Serum albumin coating of demineralized bone matrix results in stronger new bone formation. *Journal of Biomedical Materials Research Part B, Applied Biomaterials* 104(1):126–32, 2016 Jan. <https://doi.org/10.1002/jbm.b.33359> PMID: 25677203.
46. Li R, Stewart DJ, Von Schroeder HP, Mackinnon ES, Schemitsch EH. Effect of cell-based VEGF gene therapy on healing of a segmental bone defect. *Journal of Orthopaedic Research* 27 (1) (pp 8–14), 2009 Date of Publication: January 2009. 2009. <https://doi.org/10.1002/jor.20658> PMID: 18634016.
47. Matsumoto T, Sato D, Hashimoto Y. Individual and combined effects of noise-like whole-body vibration and parathyroid hormone treatment on bone defect repair in ovariectomized mice. *Proceedings of the Institution of Mechanical Engineers Part H, Journal of engineering in medicine* 230 (1) (pp 30–38), 2016 Date of Publication: 01 Jan 2016. <https://doi.org/10.1177/0954411915616987> PMID: 26586525.
48. Nam SS, Lee JC, Kim HJ, Park JW, Lee JM, Suh JY, et al. Serotonin Inhibits Osteoblast Differentiation and Bone Regeneration in Rats. *Journal of Periodontology* 87(4):461–9, 2016 Apr. <https://doi.org/10.1902/jop.2015.150302> PMID: 26693696.
49. Pigossi SC, de Oliveira GJ, Finoti LS, Nepomuceno R, Spolidorio LC, Rossa C Jr., et al. Bacterial cellulose-hydroxyapatite composites with osteogenic growth peptide (OGP) or pentapeptide OGP on bone regeneration in critical-size calvarial defect model. *Journal of Biomedical Materials Research Part A* 103(10):3397–406, 2015 Oct. <https://doi.org/10.1002/jbm.a.35472> PMID: 25850694.
50. Rai B, Chatterjea A, Lim ZXH, Tan TC, Sawyer AA, Hosaka YZ, et al. Repair of segmental ulna defects using a beta-TCP implant in combination with a heparan sulfate glycosaminoglycan variant. *Acta Biomaterialia* 28 (pp 193–204), 2015 Date of Publication: December 2015. <https://doi.org/10.1016/j.actbio.2015.09.008> PMID: 26384700.
51. Sassioto MC, Inouye CM, Aydos RD, Figueiredo AS. Bone repair in rats treated with sodic diclofenac and calcitonin. *Acta Cirurgica Brasileira*. 2006; 21 Suppl 4:40–4. PMID: 17293965.
52. Tresguerres IF, Alobera MA, Baca R, Tresguerres JA. Histologic, morphometric, and densitometric study of peri-implant bone in rabbits with local administration of growth hormone. *International Journal of Oral & Maxillofacial Implants*. 2005; 20(2):193–202. PMID: 15839112.
53. Atalay Y, Bozkurt MF, Gonul Y, Cakmak O, Agacayak KS, Kose I, et al. The effects of amlodipine and platelet rich plasma on bone healing in rats. *Drug Des Devel Ther*. 2015; 9:1973–81. <https://doi.org/10.2147/DDDT.S80778> PMID: 25897207.
54. Cao Y, Xiong J, Mei S, Wang F, Zhao Z, Wang S, et al. Aspirin promotes bone marrow mesenchymal stem cell-based calvarial bone regeneration in mini swine. *Stem Cell Research and Therapy* 6 (1) (no pagination), 2015 Article Number: 210 Date of Publication: 31 Oct 2015.
55. Durmuslar MC, Balli U, Ongoz Dede F, Bozkurt Dogan S, Misir AF, Baris E, et al. Evaluation of the effects of platelet-rich fibrin on bone regeneration in diabetic rabbits. *Journal of Cranio-Maxillo-Facial Surgery* 44(2):126–33, 2016 Feb. <https://doi.org/10.1016/j.jcms.2015.11.009> PMID: 26732635.
56. Konofaos P, Petersen D, Jennings JA, Smith RA, Doty H, Reves BT, et al. Evaluation of Amniotic Multipotential Tissue Matrix to Augment Healing of Demineralized Bone Matrix in an Animal Calvarial Model. *Journal of Craniofacial Surgery* 26(4):1408–12, 2015 Jun. <https://doi.org/10.1097/SCS.0000000000001741> PMID: 26080207.
57. Schneppendahl J, Jungbluth P, Sager M, Benga L, Herten M, Scholz A, et al. Synergistic effects of HBO and PRP improve bone regeneration with autologous bone grafting. *Injury* 47 (12) (pp 2718–

- 2725), 2016 Date of Publication: 01 Dec 2016. <https://doi.org/10.1016/j.injury.2016.09.039> PMID: 27817884.
58. Yu Z, Geng J, Gao H, Zhao X, Chen J. Evaluations of guided bone regeneration in canine radius segmental defects using autologous periosteum combined with fascia lata under stable external fixation. *Journal of Orthopaedics and Traumatology* 16 (2) (pp 133–140), 2015 Article Number: 321 Date of Publication: 12 Oct 2015. <https://doi.org/10.1007/s10195-014-0321-z> PMID: 25308901.
59. Alpan AL, Toker H, Ozer H. Ozone Therapy Enhances Osseous Healing in Rats With Diabetes With Calvarial Defects: A Morphometric and Immunohistochemical Study. *Journal of Periodontology* 87 (8):982–9, 2016 Aug. <https://doi.org/10.1902/jop.2016.160009> PMID: 26991488.
60. Calvo-Guirado JL, Garces M, Delgado-Ruiz RA, Ramirez Fernandez MP, Ferres-Amat E, Romanos GE. Biphasic beta-TCP mixed with silicon increases bone formation in critical site defects in rabbit calvaria. *Clinical Oral Implants Research* 26(8):891–7, 2015 Aug. <https://doi.org/10.1111/clr.12413> PMID: 24863557.
61. Costa NM, Yassuda DH, Sader MS, Fernandes GV, Soares GD, Granjeiro JM. Osteogenic effect of tricalcium phosphate substituted by magnesium associated with Genderm membrane in rat calvarial defect model. *Materials Science & Engineering C, Materials for Biological Applications* 61:63–71, 2016 Apr 01. <https://doi.org/10.1016/j.msec.2015.12.003> PMID: 26838825.
62. Durand M, Collombet JM, Frasca S, Begot L, Lataillade JJ, Le Bousse-Kerdiles MC, et al. In vivo hypobaric hypoxia performed during the remodeling process accelerates bone healing in mice. *Stem Cells Translational Medicine*. 2014; 3(8):958–68. <https://doi.org/10.5966/sctm.2013-0209> PMID: 24944208.
63. Elgali I, Turri A, Xia W, Norlindh B, Johansson A, Dahlin C, et al. Guided bone regeneration using resorbable membrane and different bone substitutes: Early histological and molecular events. *Acta Biomaterialia* 29:409–23, 2016 Jan. <https://doi.org/10.1016/j.actbio.2015.10.005> PMID: 26441123.
64. Grassmann JP, Schnependahl J, Hakimi AR, Herten M, Betsch M, Logters TT, et al. Hyperbaric oxygen therapy improves angiogenesis and bone formation in critical sized diaphyseal defects. *Journal of Orthopaedic Research*. 2015; 33(4):513–20. <https://doi.org/10.1002/jor.22805> PMID: 25640997.
65. Park JW, Kang DG, Hanawa T. New bone formation induced by surface strontium-modified ceramic bone graft substitute. *Oral Diseases* 22(1):53–61, 2016 Jan. <https://doi.org/10.1111/odi.12381> PMID: 26458092.
66. Uchida R, Nakata K, Kawano F, Yonetani Y, Ogasawara I, Nakai N, et al. Vibration acceleration promotes bone formation in rodent models. *PLoS ONE* 12 (3) (no pagination), 2017 Article Number: e0172614 Date of Publication: March 2017.
67. Wang G, Wang J, Fu Y, Bai L, He M, Li B, et al. Systemic treatment with vanadium absorbed by *Coprinus comatus* promotes femoral fracture healing in streptozotocin-diabetic rats. *Biological Trace Element Research*. 2013; 151(3):424–33. <https://doi.org/10.1007/s12011-012-9584-5> PMID: 23271683.
68. de Oliveira HT, Bergoli RD, Hirsch WD, Chagas OL Jr., Heitz C, Silva DN. Isotretinoin effect on the repair of bone defects—a study in rat calvaria. *Journal of Cranio-Maxillo-Facial Surgery*. 2013; 41 (7):581–5. <https://doi.org/10.1016/j.jcms.2012.11.030> PMID: 23273647.
69. Dong GC, Chen HM, Yao CH. A novel bone substitute composite composed of tricalcium phosphate, gelatin and *drynaria fortunei* herbal extract. *Journal of Biomedical Materials Research Part A*. 2008; 84(1):167–77. <https://doi.org/10.1002/jbm.a.31261> PMID: 17607749.
70. Hadzik J, Kubasiewicz-Ross P, Kunert-Keil C, Jurczynszyn K, Nawrot-Hadzik I, Dominiak M, et al. A silver carp skin derived collagen in bone defect treatment—A histological study in a rat model. *Annals of Anatomy* 208:123–128, 2016 Nov. <https://doi.org/10.1016/j.aanat.2016.07.009> PMID: 27507153.
71. Han X, Du J, Liu D, Liu H, Amizuka N, Li M. Histochemical examination of systemic administration of eldelcalcitol combined with guided bone regeneration for bone defect restoration in rats. *Journal of Molecular Histology* 48(1):41–51, 2017 Feb. <https://doi.org/10.1007/s10735-016-9705-0> PMID: 27882438.
72. Jia P, Chen H, Kang H, Qi J, Zhao P, Jiang M, et al. Deferoxamine released from poly(lactic-co-glycolic acid) promotes healing of osteoporotic bone defect via enhanced angiogenesis and osteogenesis. *Journal of Biomedical Materials Research Part A* 104(10):2515–27, 2016 Oct. <https://doi.org/10.1002/jbm.a.35793> PMID: 27227768.
73. Lee JY, Son SJ, Son JS, Kang SS, Choi SH. Bone-healing capacity of PCL/PLGA/duck beak scaffold in critical bone defects in a rabbit model. *BioMed Research International* 2016 (no pagination), 2016 Article Number: 2136215 Date of Publication: 2016.
74. Li W, Zhao Z, Xiong J, Zeng Y. The modification experimental study in vivo of nano-bone gelatin. *Artificial Cells, Nanomedicine, & Biotechnology*. 2014; 42(5):309–15. <https://doi.org/10.3109/21691401.2013.821411> PMID: 23899020.

75. Ngueguim FT, Khan MP, Donfack JH, Siddiqui JA, Tewari D, Nagar GK, et al. Evaluation of Cameroonian plants towards experimental bone regeneration. *Journal of Ethnopharmacology*. 2012; 141(1):331–7. <https://doi.org/10.1016/j.jep.2012.02.041> PMID: 22414477.
76. Ngueguim FT, Khan MP, Donfack JH, Tewari D, Dimo T, Kamtchouing P, et al. Ethanol extract of *Peperomia pellucida* (Piperaceae) promotes fracture healing by an anabolic effect on osteoblasts. *Journal of Ethnopharmacology*. 2013; 148(1):62–8. <https://doi.org/10.1016/j.jep.2013.03.063> PMID: 23578859.
77. Zhao X, Wu ZX, Zhang Y, Gao MX, Yan YB, Cao PC, et al. Locally administrated perindopril improves healing in an ovariectomized rat tibial osteotomy model. *PLoS ONE [Electronic Resource]*. 2012; 7(3): e33228. <https://doi.org/10.1371/journal.pone.0033228> PMID: 22427998.
78. Bougioukli S, Jain A, Sugiyama O, Tinsley BA, Tang AH, Tan MH, et al. Combination therapy with BMP-2 and a systemic RANKL inhibitor enhances bone healing in a mouse critical-sized femoral defect. *Bone*. 2016; 84 (pp 93–103), 2016. Date of Publication: March 01. <https://doi.org/10.1016/j.bone.2015.12.052> PMID: 26723577.
79. Cheng BH, Chu TMG, Chang C, Kang HY, Huang KE. Testosterone Delivered with a Scaffold Is as Effective as Bone Morphologic Protein-2 in Promoting the Repair of Critical-Size Segmental Defect of Femoral Bone in Mice. *PLoS ONE* 8 (8), 2013 Article Number: e70234 Date of Publication: 05 Aug 2013. 2013.
80. Gocer H, Onger ME, Kuyubasi N, Cirakli A, Kir MC. The effect of teicoplanin on fracture healing: an experimental study. *Eklemler hastaliklari ve cerrahisi = Joint Diseases & Related Surgery* 27(1):16–21, 2016. PMID: 26874630.
81. Kanczler JM, Ginty PJ, Barry JJA, Clarke NMP, Howdle SM, Shakesheff KM, et al. The effect of mesenchymal populations and vascular endothelial growth factor delivered from biodegradable polymer scaffolds on bone formation. *Biomaterials* 29 (12) (pp 1892–1900), 2008 Date of Publication: April 2008. 2008. <https://doi.org/10.1016/j.biomaterials.2007.12.031> PMID: 18234329.
82. Ma X, Wang Y, Guo H, Wang J. Nano-hydroxyapatite/chitosan sponge-like biocomposite for repairing of rat calvarial critical-sized bone defect. *Journal of Bioactive and Compatible Polymers* 26 (4) (pp 335–346), 2011 Date of Publication: July 2011. 2011.
83. Myers TJ, Yan Y, Granero-Molto F, Weis JA, Longobardi L, Li T, et al. Systemically delivered insulin-like growth factor-I enhances mesenchymal stem cell-dependent fracture healing. *Growth Factors* 30 (4) (pp 230–241), 2012 Date of Publication: August 2012. 2012. <https://doi.org/10.3109/08977194.2012.683188> PMID: 22559791.
84. Toupadakis CA, Granick JL, Sagy M, Wong A, Ghassemi E, Chung DJ, et al. Mobilization of endogenous stem cell populations enhances fracture healing in a murine femoral fracture model. *Cytotherapy*. 2013; 15(9):1136–47. <https://doi.org/10.1016/j.jcyt.2013.05.004> PMID: 23831362.
85. Wang Y, Lv P, Ma Z, Zhang J. Enhanced healing of rat calvarial critical size defect with selenium-doped lamellar biocomposites. *Biological Trace Element Research* 155 (1) (pp 72–81), 2013 Date of Publication: October 2013. 2013. <https://doi.org/10.1007/s12011-013-9763-z> PMID: 23892698.
86. Yoshii T, Nyman JS, Yuasa M, Esparza JM, Okawa A, Gutierrez GE. Local application of a protease-inhibitor enhances fracture healing in rats. *Journal of Orthopaedic Research*. 2015; 33(8):1197–204. <https://doi.org/10.1002/jor.22849> PMID: 25683968.
87. Asutay F, Polat S, Gul M, Subasi C, Kahraman SA, Karaoz E. The effects of dental pulp stem cells on bone regeneration in rat calvarial defect model: micro-computed tomography and histomorphometric analysis. *Arch Oral Biol*. 2015; 60(12):1729–35. <https://doi.org/10.1016/j.archoralbio.2015.09.002> PMID: 26433189.
88. Dixit M, Raghuvarshi A, Gupta CP, Kureel J, Mansoori MN, Shukla P, et al. Medicarpin, a Natural Pterocarpan, Heals Cortical Bone Defect by Activation of Notch and Wnt Canonical Signaling Pathways. *PLoS ONE* 10 (12) (no pagination), 2015 Article Number: e0144541 Date of Publication: 01 Dec 2015.
89. Jackson RA, McDonald MM, Nurcombe V, Little DG, Cool SM. The use of heparan sulfate to augment fracture repair in a rat fracture model. *Journal of Orthopaedic Research*. 2006; 24(4):636–44. <https://doi.org/10.1002/jor.20103> PMID: 16514633.
90. Matsubara H, Tsuchiya H, Watanabe K, Takeuchi A, Tomita K. Percutaneous nonviral delivery of hepatocyte growth factor in an osteotomy gap promotes bone repair in rabbits: A preliminary study. *Clinical Orthopaedics and Related Research* 466 (12) (pp 2962–2972), 2008 Date of Publication: December 2008. 2008. <https://doi.org/10.1007/s11999-008-0493-z> PMID: 18813894.
91. Sisask G, Marsell R, Sundgren-Andersson A, Larsson S, Nilsson O, Ljunggren O, et al. Rats treated with AZD2858, a GSK3 inhibitor, heal fractures rapidly without endochondral bone formation. *Bone* 54 (1) (pp 126–132), 2013 Date of Publication: May 2013. 2013. <https://doi.org/10.1016/j.bone.2013.01.019> PMID: 23337038.

92. Alic T, Cirakli A, Sahin Y, Tomak Y. Effects of cilostazol on fracture healing: an experimental study. *Acta Orthop Traumatol Turc.* 2016; 50(1):103–10. <https://doi.org/10.3944/AOTT.2016.15.0211> PMID: 26854057.
93. Baht GS, Nadesan P, Silkstone D, Alman BA. Pharmacologically targeting beta-catenin for NF1 associated deficiencies in fracture repair. *Bone* 98 (pp 31–36), 2017 Date of Publication: 01 May 2017. <https://doi.org/10.1016/j.bone.2017.02.012> PMID: 28254468.
94. Bernick J, Wang Y, Sigal IA, Alman BA, Whyne CM, Nam D. Parameters for lithium treatment are critical in its enhancement of fracture-healing in rodents. *Journal of Bone & Joint Surgery—American Volume.* 2014; 96(23):1990–8. <https://doi.org/10.2106/JBJS.N.00057> PMID: 25471914.
95. Cai Y, Guo L, Shen H, An X, Jiang H, Ji F, et al. Degradability, bioactivity, and osteogenesis of biocomposite scaffolds of lithium-containing mesoporous bioglass and mPEG-PLGA-b-PLL copolymer. *International Journal of Nanomedicine* 10:4125–36, 2015. <https://doi.org/10.2147/IJN.S82945> PMID: 26150718.
96. Cakmak G, Sahin MS, Ozdemir BH, Karadeniz E. Effect of pentoxifylline on healing of segmental bone defects and angiogenesis. *Acta orthopaedica et traumatologica turcica* 49 (6) (pp 676–682), 2015 Date of Publication: 2015. <https://doi.org/10.3944/AOTT.2015.15.0158> PMID: 26511696.
97. Del Rosario C, Rodriguez-Evora M, Reyes R, Simoes S, Concheiro A, Evora C, et al. Bone critical defect repair with poloxamine-cyclodextrin supramolecular gels. *International Journal of Pharmaceutics* 495 (1) (pp 463–473), 2015 Date of Publication: 10 Nov 2015. <https://doi.org/10.1016/j.ijpharm.2015.09.003> PMID: 26362078.
98. Donneys A, Weiss DM, Deshpande SS, Ahsan S, Tchanque-Fossuo CN, Sarhaddi D, et al. Localized deferoxamine injection augments vascularity and improves bony union in pathologic fracture healing after radiotherapy. *Bone.* 2013; 52(1):318–25. <https://doi.org/10.1016/j.bone.2012.10.014> PMID: 23085084.
99. Fan J, Guo M, Im CS, Pi-Anfruns J, Cui ZK, Kim S, et al. Enhanced Mandibular Bone Repair by Combined Treatment of Bone Morphogenetic Protein 2 and Small-Molecule Phenamil. *Tissue Engineering—Part A* 23 (5–6) (pp 195–207), 2017 Date of Publication: March 2017. <https://doi.org/10.1089/ten.TEA.2016.0308> PMID: 27771997.
100. Ishack S, Mediero A, Wilder T, Ricci JL, Cronstein BN. Bone regeneration in critical bone defects using three-dimensionally printed beta-tricalcium phosphate/hydroxyapatite scaffolds is enhanced by coating scaffolds with either dipyridamole or BMP-2. *Journal of Biomedical Materials Research—Part B Applied Biomaterials* 105 (2) (pp 366–375), 2017 Date of Publication: 01 Feb 2017.
101. Kutan E, Duygu-Capar G, Ozcaker-Tomruk C, Dilek OC, Ozen F, Erdogan O, et al. Efficacy of doxycycline release collagen membrane on surgically created and contaminated defects in rat tibiae: A histopathological and microbiological study. *Archives of Oral Biology* 63:15–21, 2016 Mar. <https://doi.org/10.1016/j.archoralbio.2015.11.001> PMID: 26658367.
102. Limirio PHJO, Rocha FS, Batista JD, Guimaraes-Henriques JC, de Melo GB, Dechichi P. The Effect of Local Delivery Doxycycline and Alendronate on Bone Repair. *AAPS PharmSciTech* 17 (4) (pp 872–877), 2016 Date of Publication: 01 Aug 2016. <https://doi.org/10.1208/s12249-015-0411-0> PMID: 26381914.
103. Wada K, Yu W, Elazizi M, Barakat S, Ouimet MA, Rosario-Melendez R, et al. Locally delivered salicylic acid from a poly(anhydride-ester): impact on diabetic bone regeneration. *Journal of Controlled Release.* 2013; 171(1):33–7. <https://doi.org/10.1016/j.jconrel.2013.06.024> PMID: 23827476.
104. Werkman C, Senra GS, da Rocha RF, Brandao AA. Comparative therapeutic use of Risedronate and Calcarea phosphorica—allopathy versus homeopathy—in bone repair in castrated rats. *Pesquisa Odontologica Brasileira = Brazilian Oral Research.* 2006; 20(3):196–201. PMID: 17119700.
105. Wixted JJ, Fanning PJ, Gaur T, O'Connell SL, Silva J, Mason-Savas A, et al. Enhanced fracture repair by leukotriene antagonism is characterized by increased chondrocyte proliferation and early bone formation: a novel role of the cysteinyl LT-1 receptor. *Journal of Cellular Physiology.* 2009; 221(1):31–9. <https://doi.org/10.1002/jcp.21809> PMID: 19544365.
106. Kilkenny C, Parsons N, Kadoszewski E, Festing MF, Cuthill IC, Fry D, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PloS one.* 2009; 4 (11):e7824. <https://doi.org/10.1371/journal.pone.0007824> PMID: 19956596
107. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR journal.* 2002; 43(4):244–58. PMID: 12391400
108. Festing MF, Overend P, Gaines Das R, Cortina-Borja M, Berdoy M. The design of animal experiments: reducing the use of animals in research through better experimental design: The Royal Society of Medicine Press Limited; 2002.
109. McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C. Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *British journal of pharmacology.* 2010; 160(7):1573–6. <https://doi.org/10.1111/j.1476-5381.2010.00873.x> PMID: 20649560

110. Olsen CH. Review of the use of statistics in infection and immunity. *Infection and immunity*. 2003; 71(12):6689–92. <https://doi.org/10.1128/IAI.71.12.6689-6692.2003> PMID: 14638751
111. Altman DG, Bland JM. Standard deviations and standard errors. *Bmj*. 2005; 331(7521):903. <https://doi.org/10.1136/bmj.331.7521.903> PMID: 16223828
112. Marsh J, Slongo TF, Agel J, Broderick JS, Creevey W, DeCoster TA, et al. Fracture and dislocation classification compendium-2007: Orthopaedic Trauma Association classification, database and outcomes committee. LWW; 2007.
113. Reichert JC, Saifzadeh S, Wulschleger ME, Epari DR, Schütz MA, Duda GN, et al. The challenge of establishing preclinical models for segmental bone defect research. *Biomaterials*. 2009; 30(12):2149–63. <https://doi.org/10.1016/j.biomaterials.2008.12.050> PMID: 19211141
114. de Vries R, Wever KE, Avey MT, Stephens ML, Sena ES, Leenaars M. The usefulness of systematic reviews of animal experiments for the design of preclinical and clinical studies. *ILAR journal*. 2014; 55(3):427–37. <https://doi.org/10.1093/ilar/ilu043> PMID: 25541545
115. Van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. Can animal models of disease reliably inform human studies? *PLoS medicine*. 2010; 7(3):e1000245. <https://doi.org/10.1371/journal.pmed.1000245> PMID: 20361020
116. O'Loughlin PF, Morr S, Bogunovic L, Kim AD, Park B, Lane JM. Selection and development of preclinical models in fracture-healing research. *JBJS*. 2008; 90(Supplement_1):79–84.
117. Hackam DG, Redelmeier DA. Translation of research evidence from animals to humans. *Jama*. 2006; 296(14):1727–32.
118. ter Riet G, Korevaar DA, Leenaars M, Sterk PJ, Van Noorden CJ, Bouter LM, et al. Publication bias in laboratory animal research: a survey on magnitude, drivers, consequences and potential solutions. *PLoS One*. 2012; 7(9):e43404. <https://doi.org/10.1371/journal.pone.0043404> PMID: 22957028

